201-14906B

ROBUST SUMMARY
OF INFORMATION ON

201-16126B

**Substance Group:** **RECLAIMED SUBTANCES: NAPHTHENIC ACID**



**Summary prepared by:** **American Petroleum Institute**

**Date of last Update:** **DECEMBER 15, 2003**

**Number of pages:** **40**

Reliability of data included in this summary has been assessed using the approache described by Klimisch, et al.

Klimisch, H.J., Andreae, M. and Tillman, U (1997)
A systemic approach for evaluating the quality of experimental toxicological and exotoxicological data.
Regulatory Toxicology and Pharmacology 25: 1-5

# 1. General Information

## 1.1 GENERAL SUBSTANCE INFORMATION

**Substance Type:** Naphthenic Acids

**Physical status:** Naphthenic acid fractions are oily liquids. The salts may be liquid or solid. Naphthenic acids (CASRN 1338-24-5) are classified as monobasic carboxylic acids of the general formula RCOOH, where R represents the naphthene moiety consisting of cyclopentane and cyclohexane derivatives. Naphthenic acids are composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids, with smaller amounts of acyclic aliphatic acids. The cycloaliphatic acids include single and fused multiple cyclopentane and cyclohexane rings. The carboxyl group is usually attached to a side chain rather than directly to the ring. Aromatic, olefinic, hydroxy and dibasic acids are present as minor components.

Naphthenic acids recovered from refinery streams occur naturally in the crude oil and are not formed during the refining process. Heavy crudes have the highest acid content, and paraffinic crudes usually have low acid content. Naphthenic acids are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions.

# 2. Physical and Chemical Data

## 2.1 MELTING POINT

**Test Substance:** Naphthenic Acids, commercial mixtures

**Method:** Not stated

**Year (Guideline):** Not stated

**Type (test type):** Not stated

**GLP:** Unknown

**Test Conditions:** Unknown

**Results:**

| | |
|---|---|
| -35 °C to +0 °C | Ref (1) |
| -35 °C to +2 °C | Ref (2) |
| +30 °C | Ref (3) |

**Remark:** Values cited represent ranges of melting points cited in product literature data and Material Safety Data Sheet for commercial naphthenic acid products.

**Source:**

(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

(2) AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

(3) Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

**Reliability:** (4) Not assignable. Original source data were not available for review.

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method/Guideline:** Calculated values using MPBPWIN Version 1.40, a subroutine of the computer program EPIWIN Version 3.10

**Year (guideline):** 2000

**Type (test type):** Not applicable

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

**Results:**

| Naphthenic Acid Type | Carbon Number | Molecular Weight | Melting Point, °C |
|---|---|---|---|
| 1-ring cyclopentane | 16 | 254 | 117 |
| 1-ring cyclohexane | 21 | 325 | 155 |
| 2-ring cyclopentane | 17 | 266 | 127 |
| 2-ring cyclohexane | 21 | 323 | 157 |
| 3-ring cyclohexane | 17 | 264 | 128 |
| 3-ring cyclohexane | 21 | 321 | 160 |
| 4-ring cyclohexane | 17 | 262 | 131 |
| 4-ring cyclohexane | 21 | 319 | 156 |

**Remark:**

Substances in this category do not have a specific melting point but a range of melting points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual melting point ranges will vary dependent upon their constituent composition.

Melting point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated melting points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products, as evidenced by comparing the estimated melting point values to those cited in product literature and MSDS data (SocTech, S.A. 2003; AGS Chemicals Limited. 2003; Mallinckrodt Baker, Inc. 1997).

**Source:**

U.S. EPA. 2000. API (Estimation programs interface) suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

**Reliability:**

(2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of melting point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

## 2.2 BOILING POINT

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Not stated

**Year:** Not stated

**Type:** Not stated

**GLP:** Not stated

**Year (study performed):** Not stated

**Test Conditions:** Not stated

**Results:**

| | |
|---|---|
| 250 °C to 350 °C | Ref (1) |
| 140 °C to 200 °C | Ref (2) |
| 200 °C to 370 °C | Ref (3) |

**Remark:** Values reported vary widely due to varied composition of the hydrocarbon mixture in naphthenic acids. Values given represent various commercial preparations of naphthenic acids.

**Source:**

(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

(2) AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

(3) Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic Acids. In: Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc.

**Reliability:** (4) Not assignable

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN©, MPBPWIN V1.40 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

**Results:** Boiling point values for various cycloaliphatic carboxylic acids in naphthenic acid mixtures are:

| Compound | Estimated Boiling Point, °C |
|---|---|
| C7 cyclohexane | 233 |
| C9 dicyclopentane | 266 |
| C10 cyclopentane | 284 |
| C11 cyclohexane | 301 |
| C13 dicyclopentane | 326 |
| C14 cyclopentane | 340 |
| C15 cyclohexane | 352 |
| C17 dicyclopentane | 373 |
| C17 tricyclohexane | 375 |

**Remark:** Substances in this category do not have a specific boiling point but a range of boiling points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual boiling point ranges will vary dependent upon their constituent composition.

Boiling point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated boiling points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

**Reliability:** (2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of boiling point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

## 2.4 VAPOR PRESSURE

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN©, MPBPWIN V1.40 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, vapor pressures were calculated by MPBPWIN, V1.40, EPIWIN V3.10

**Results:** Estimated vapor pressures for various naphthenic acid compounds:

| Naphthenic Acid Type | Carbon Number | Molecular Weight | Vapor Pressure, Pa |
|---|---|---|---|
| 1-ring cyclopentane | 16 | 254 | $1.8 \times 10^{-3}$ |
| 1-ring cyclohexane | 21 | 325 | $1.5 \times 10^{-5}$ |
| 2-ring cyclopentane | 17 | 266 | $4.8 \times 10^{-4}$ |
| 2-ring cyclohexane | 21 | 323 | $1.5 \times 10^{-5}$ |
| 3-ring cyclohexane | 17 | 264 | $4.2 \times 10^{-4}$ |
| 3-ring cyclohexane | 21 | 321 | $1.4 \times 10^{-5}$ |
| 4-ring cyclohexane | 17 | 262 | $1.6 \times 10^{-5}$ |
| 4-ring cyclohexane | 21 | 319 | $4.4 \times 10^{-4}$ |

**Remark:** A search for pressure values of naphthenic acids failed to uncover reliable information. Product literature data provided narrative phrases such as "very low" or "not applicable" when describing the vapor pressure characteristic for commercial products (SocTech, S.A., 2003; AGS Chemicals Limited. 2003). To gain an understanding of vapor pressure characteristics of naphthenic acids, various hydrocarbon acidic structures reported by Rogers et al. (2002) to predominate in naphthenic acids were estimated for vapor pressure using the EPIWIN© computer model (U.S. EPA 2000).

The vapor pressure of complex mixtures is equal to the sum of the vapor pressures of the individual constituents in their pure form times their mole fraction in the mixture (Raoult's Law). Therefore, the total vapor pressure of a complex mixture of naphthenic acids will depend on the proportion of different molecular weight constituents making up the mixture. It is estimated from vapor pressure modeling that representative individual naphthenic acid molecules will have vapor pressure values near or below the measurable limits cited in standard reference guidelines (OECD Guideline 104, Vapor Pressure; OECD, 1995). Hence, based on Raoult's Law, the total vapor pressure of naphthenic acids is expected to be exceedingly low.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

OECD (Organization for Economic Cooperation and Development). 1995. OECD Guideline 104, Vapor Pressure. OECD, Paris, France.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

**Reliability:** (2) Reliable with restrictions
Estimated vapor pressures were obtained from a validated computer program.

## 2.5 PARTITION COEFFICIENT

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN©, KOWWIN V1.66 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, vapor pressures were calculated by KOWWIN, V1.66, EPIWIN V3.10

**Results:** Tabulated values for various naphthenic acid molecules are:

| Naphthenic Acid Type | Carbon Number | Molecular Weight | Log Kow |
|---|---|---|---|
| 1-ring cyclopentane | 16 | 254 | 6.7 |
| 1-ring cyclohexane | 21 | 325 | 9.2 |
| 2-ring cyclopentane | 17 | 266 | 6.3 |
| 2-ring cyclohexane | 21 | 323 | 8.3 |
| 3-ring cyclohexane | 17 | 264 | 5.4 |
| 3-ring cyclohexane | 21 | 321 | 7.3 |
| 4-ring cyclohexane | 17 | 262 | 6.5 |
| 4-ring cyclohexane | 21 | 319 | 5.1 |

**Remark:** No partition coefficient measurements were found for naphthenic acids. Therefore, partition coefficients for a range of molecular weight naphthenic acids were estimated using the EPIWIN© computer model (U.S. EPA 2000). The partition coefficients reported here span the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected, however, that the lowest molecular weight structures will have the lowest partition coefficients of the compounds in the complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures of molecular weights below 250 will likely show log Kow values lower than those estimated here.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

**Reliability:** (2) Reliable with restrictions
Estimated vapor pressures were obtained from a validated computer program.

## 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in:** Water

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN©, WSKOWWIN V1.40 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, water solubility values were calculated by WSKOWWIN, V1.40, EPIWIN V3.10

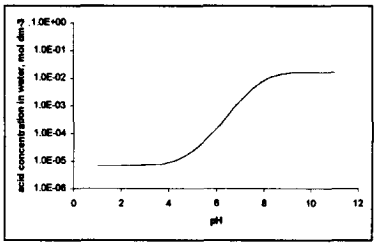
**Results:** Tabulated estimates at 25°C for various naphthenic acid molecular structures are:

| Naphthenic Acid Type | Carbon Number | Molecular Weight | Water Solubility, mg/l |
|---|---|---|---|
| 1-ring cyclopentane | 16 | 254 | 0.11 |
| 1-ring cyclohexane | 21 | 325 | 0.0003 |
| 2-ring cyclopentane | 17 | 266 | 0.19 |
| 2-ring cyclohexane | 21 | 323 | 0.002 |

| | | | |
|---|---|---|---|
| 3-ring cyclohexane | 17 | 264 | 1.2 |
| 3-ring cyclohexane | 21 | 321 | 0.01 |
| 4-ring cyclohexane | 17 | 262 | 0.08 |
| 4-ring cyclohexane | 21 | 319 | 2.1 |

**Remark:**

No water solubility measurements were found for naphthenic acids, but their dissociation equilibrium in aqueous systems provides a general understanding of their behavior. These compounds exist as weak acids, with most pKa values being reported at about 5 (Havre, 2002). At low pHs, these compounds exist in their undissociated form and tend to partition onto solids (Rogers et al., 2002). At high pHs, they exist in their dissociated form and become more mobile (Havre, 2002). The following plot shows a theoretical model of the concentration of the acid in the water phase with water pH. This relationship is used as the basis for extraction of naphthenic acids from crude oil, where an alkaline hot water extraction process is used (CEATAG 1998; Brient et al., 1995). However, solubility does not follow an exact acid/base equilibrium, and the equilibrium between oil and water becomes increasingly complex as pH rises. This is due to the tendency of these substances to form micelles and reversed micelles at alkaline pHs. In this system, the existence of 4 or 5 isotropic phases can be observed, making exact solubility measurements difficult (Havre, 2002).



from Havre, 2002

To gain an overview of the water solubility of a range of molecular weight naphthenic acids, the EPIWIN© computer model (U.S. EPA 2000) was used to generate solubility estimates for different molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected that the lowest molecular weight structures will have the greatest water solubility of the compounds in complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures having molecular weights below 250 will likely show water solubilities greater than those estimated here.

**Source:**

U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Havre, T.E. 2002. Formation of calcium naphthenate in water/oil systems, naphthenic acid chemistry and emulsion stability. Ph.D. Thesis, Department of Chemical Engineering, Norwegian University of Science and Technology, Trondheim, Norway. October 2002.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic acids. In: Kroschwitz, J.I. (ed.). Encyclopedia of Chemical Technology, Vol. 16, 4th ed. John Wiley & Sons, Inc., New York. pp 1017 – 1029.

**Reliability:** (2) Reliable with restrictions
Estimated water solubility values were obtained from a validated computer program.

## 2.14 ADDITIONAL REMARKS

**Memo:** Water solubility of naphthenic acids

**Remark:** Values of water solubility reported in product literature data have varied widely. CEATAG (1998) reported water solubility values of one commercial product to range from 70 mg/l at pH 0.91 to 5040 mg/l at pH 9.16. Other product data sources for water solubility report narrative phrases such as "very low water solubility" (SocTech S.A., 2003), "not applicable" (Mallinckrodt Baker Inc., 1997), or "only slightly soluble in water" (AGS Chemicals Limited, 2003).

**Source:** CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

**Reliability:** (4) Not assignable. Data were obtained from secondary literature sources.

# 3. Environmental Fate Data

## 3.1.1 PHOTODEGRADATION

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculations by EPIWIN© V3.10; Subroutine AOPWIN V1.90.

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, photodegradation potential was calculated by AOPWIN, V1.90, EPIWIN V3.10

**Results:**

| Type | Carbon Number | Molecular Weight | Half Life (days) |
|---|---|---|---|
| 1-ring cyclopentane | 16 | 254 | 0.6 |
| 1-ring cyclohexane | 21 | 325 | 0.4 |
| 2-ring cyclopentane | 17 | 266 | 0.5 |
| 2-ring cyclohexane | 21 | 323 | 0.3 |
| 3-ring cyclohexane | 17 | 264 | 0.3 |
| 3-ring cyclohexane | 21 | 321 | 0.3 |
| 4-ring cyclohexane | 17 | 262 | 0.3 |
| 4-ring cyclohexane | 21 | 319 | 0.3 |

**Remark:** AOPWIN V1.90 calculates atmospheric oxidation rate constants between photochemically produced hydroxyl radicals and organic chemicals. These rate constants are then used to calculate half lives for those compounds based on average atmospheric concentrations of hydroxyl radicals and ozone. Atmospheric oxidation rates were calculated for a range of molecular structures covering a range of molecular weights and ring structures that were reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002).

Although the low vapor pressures of these base oils indicate that volatilization will not be a very significant fate process, oxidation half-lives indicate that any vapors emitted to the troposphere would be rapidly oxidized and not persist in the atmosphere.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

**Reliability:** (2) Reliable with restrictions
Estimated water solubility values were obtained from a validated computer program.

## 3.1.2 STABILITY IN WATER

**Remark:** Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). The chemical components found in the materials that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

**Source:** Harris, J.C. 1982. Rate of hydrolysis. In; Handbook of Chemical Property Estimation Methods. W.L. Lyman, W.F. Reehl, and D.H. Rosenblastt, eds. Mcgraw-Hill Book Co., New York, NY.

## 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Level 1 Fugacity-Based Environmental Equilibrium Partitioning Model (Version 2.11)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment.

**Results:** Air / Water / Soil / Sediment / Suspended Sediment / Biota

**Type (C-number)(Molecular Weight)**

**Distribution In:**

| Air | Water | Soil | Sediment | Suspended Sediment | Biota |
|---|---|---|---|---|---|
| 1-ring cyclopentane (C16)(254) | | | | | |
| <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| 1-ring cyclohexane (C21)(325) | | | | | |
| <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| 2-ring cyclopentane (C17)(266) | | | | | |
| <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| 2-ring cyclohexane (C21)(323) | | | | | |
| <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| 3-ring cyclohexane (C17)(264) | | | | | |
| <0.1 | 0.4 | 97 | 2 | <0.1 | <0.1 |
| 3-ring cyclohexane (C21)(321) | | | | | |
| <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| 4-ring cyclohexane (C17)(262) | | | | | |
| <0.1 | <0.1 | 98 | 2 | <0.1 | <0.1 |
| 4-ring cyclohexane (C21)(319) | | | | | |

**Remark:** Multimedia distribution was calculated for a range of naphthenic acids covering predominant molecular weight and ring structures of such constituents found in Athabasca oil sands extracts (Rogers et al., 2002). The principle distribution of these constituents following an environmental release would be to soil and/or sediment, with overwhelming partitioning to soil.

**Source:** Mackay, D. 1991. Multimedia environmental models; The fugacity approach Lewis Publ. CRC Press, Boca Raton, Florida.

**Reliability:** (2) Reliable with restrictions
Estimated environmental distribution was obtained from a validated computer program.

## 3.5 BIODEGRADATION

**Remark:** No standardized testing for ready or inherent biodegradation was found for naphthenic acids. Results of relevant scientific journal articles on the biodegradability of naphthenic acids are reviewed in Section 3.8

## 3.8 ADDITIONAL REMARKS

**Memo:** Biodegradation of naphthenic acids

**Remark:** Herman et al. (1993) conducted four experiments on the biodegradation of specific cycloalkane carboxylic acids:

**Experiment No. 1.** Biodegradation of four naphthenic acid compounds (cyclopentane carboxylic acid, CCP; cyclohexane carboxylic acid, CCH; 1-methyl-1-cyclohexane carboxylic acid, 1MCCH; and 2-methyl-1-

cyclohexane carboxylic acid, 2MCCH) was measured in pore water from Athabasca oil sands tailings ponds. The purpose of the tailings ponds was to serve as a settling basin to separate solids from liquid generated during the extraction of acidic compounds from bitumen. Therefore, the tailings ponds were considered to harbor indigenous microorganisms adapted to naphthenic acids. The collected pore water was centrifuged and filtered and served as the nutrient medium. Inoculum was 0.5 ml of the original oil sands tailings sample. Duplicate flasks containing 30 ml of medium were spiked with 1-ml aliquots of stock solutions of the different naphthenic acids to achieve a final concentration of 1000 mg/l. Test flasks received the inoculum and control flasks received inoculum in which the microbes had been heat-killed. One set of duplicate flasks received a nutrient addition in the form of $NH_4NO_3$, $K_2HPO_4$, and $KH_2PO_4$ to a final concentration of 0.2 g/l of each compound. The flasks were incubated at room temperature on a rotary shaker. After 0, 3, 6, 9, 16, 26, and 40 days, a 3-ml sample was removed, centrifuged, and filtered through a 0.2 micron syringe filter. The samples were analyzed for the test compounds by gas chromatography equipped with a flame ionization detector. Peak areas were converted to concentration using a calibration curve for each compound.

**Results of Experiment 1.** The bacterial populations of oil sands tailings was shown to have the metabolic capability of degrading carboxylated cycloalkanes as shown in the following table of results.

| | Percent Remaining | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CCP | | CCH | | 1MCCH | | 2MCCH | |
| Day | NP- | NP+ | NP- | NP+ | NP- | NP+ | NP- | NP+ |
| 0 | 100 | 42 | 100 | 68 | 100 | 100 | 100 | 100 |
| 6 | 100 | 5 | 100 | 12 | 100 | 100 | 100 | 100 |
| 10 | 100 | 0 | 100 | 1 | 100 | 100 | 100 | 100 |
| 16 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 100 |
| 26 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 49 |
| 40 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 0 |

Using tailings pond water as a growth medium, degradation of CCP, CCH, and 2MCCH was achieved only if nutrients were added to the medium. CCP and CCH were degraded rapidly, within one week, while methylated carboxylic acids were more resistant to biodegradation. 2MCCH was degraded within 40 days, but no degradation was observed for 1MCCH.

**Experiment No. 2.** Triplicate tailings pond microcosms were created using 200 ml of the tailings sample (as inoculum and medium) in 500-ml Erlenmeyer flasks closed with cotton stoppers. A filter-sterilized solution of CCP and 1MCCH was added to each microcosm for a final concentration of 1000 mg/l. Sterile controls were autoclaved and also spiked with the test compounds. Microcosms were incubated at room temperature on a rotary shaker. After 1, 2, 3, 4, 6, and 9 weeks, samples were removed and analyzed for CCP and 1MCCH by GC.

**Results of Experiment No. 2.** Biodegradation of CCP was complete within the first week. No biodegradation of 1MCCH was evident after six weeks. At the six-week period, nitrogen and phosphorus was added

whereby complete biodegradation of 1MCCH was noted following between the 6 and 9-week sampling. No 1MCCH was measured at 9 weeks. Neither CCP nor 1MCCH was degraded in the control microcosms.

**Experiment No. 3:** Tailings pond bacteria were isolated on agar plates and colony types were examined for their ability to utilize carboxylated cycloalkanes as their sole carbon source. Individual colonies were inoculated into a solution of carboxylated cycloalkanes (1000 mg/l) in modified Bushnell and Haas (MGH) minimal salts medium. The ability of the isolate to metabolize the carbon source was monitored by GC analysis. In a second part to this experiment, a carboxylate-degrading mixed bacterial culture was enriched from the tailings pond sample using standard procedures. The mixed culture was maintained on a mixture of CCP, 1MCCH, and 2MCCH (500 mg/l each) in MBH with yeast extract (1000 mg/l) added as a supplemental carbon source.

**Results of Experiment No. 3.** Of 10 separate colony types isolated from oil sands tailings, one colony type was found to utilize CCP and CCH as its sole carbon source. The isolate was a Gram negative, non-motile, catalase positive, oxidase negative, non-fermenting, aerobic rod, and was identified as an *Acinetobacter* sp. The isolate rapidly degraded CCP and CCH, with complete loss of substrate from the medium within 2 weeks of incubation. However, this isolate was unable to degrade methyl-substituted cyclohexane carboxylic acids. The mixed bacterial culture enriched from the tailings pond sample on a mixture of carboxylated cycloalkanes was found to degrade 1MCCH and 2MCCH, but only when the medium was supplemented with yeast extract. After a 2-week incubation period, the mixed culture had degraded 100% of the 1MCCH and 67% of the 2MCCH.

**Experiment No. 4.** Radiolabeled hexadecane was spiked into the maltene fraction of pure bitumen. Hexadecane mineralization experiments were performed using 5 ml of oil sands tailings in 60-ml serum vials and inoculated with 10 ul of spiked maltene. One set of vials received nutrient addition as described before. Sterile controls were autoclaved before the addition of the labeled hydrocarbon. Mineralization was determined from triplicate vials after 5, 10, 16, 27, and 40 days using the closed-loop trapping system. Radioactivity was measured using a scintillation cocktail and a Beckman LS8000 scintillation counter.

**Results of Experiment No. 4.** The results of hexadecane mineralization within oil sands tailings showed that the biodegradation of an n-alkane was nutrient limited. Percent biodegradation reached 50% by day 16 and maintained a plateau through day 40.

**Conclusions.** This study showed the potential for biodegradation of naphthenic acids by investigating the biodegradation of both carboxylated cycloalkanes and hexadecane. Although natural naphthenic acids present in oil sands tailings have greater structural complexity than the compounds examined in this study, the results show the potential for both for biodegradation of the alkyl side chain and the carboxylated cycloalkane ring components of naphthenic acids. Biodegradation potential was reduced by methyl substitution on the

cycloalkane ring, although these compounds could be degraded with the addition of mineral nutrients.

**Source:** Herman, D.C., P.M. Fedorak, and J.W. Costerton. 1993. Biodegradation of cycloalkane carboxylic acids in oil sand tailings. Can. J. Microbiol. 39:576-580.

**Reliability:** (2) Reliable with restrictions. The report was a well-documented study that meets basic scientific principles.

**Memo:** Biodegradation of cycloalkane carboxylic acids in oil sand tailings

**Remark:** Herman et al. (1994) investigated the ability of microbial populations indigenous to oil sands tailings to biodegrade solutions of natural naphthenic acids from oil sands tailings and commercial naphthenic acid sodium salts (Kodak Chemicals).

Four experiments were run:
1) Evaluation of mineralizaton of naphthenic acids sodium salts (NAS) and oil sands tailings extracts of naphthenic acids (TEX),
2) Evaluation of mineralization of four model naphthenic acid compounds, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA) 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and *trans*-4-pentylcyclohexane carboxylic acid (4PCCA),
3) Gas chromatographic analysis of NAS and TEX biodegradation, and
4) Respirometry measurements of cyclohexane pentanoic acid, NAS, and TEX in tailings microcosms.

**Test Substances:** Test substances used in the four experiments included the following materials: 1) Tailings water extract (TEX), 2) commercial sodium naphthenate mixture (NAS), and 3) pure compound naphthenic acids, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA), 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and *trans*-4-pentylcyclohexane carboxylic acid (4PCCA).

**Inoculum:** Inoculum used in the biodegradation experiments was NAS- and TEX- degrading enrichment cultures derived from oil sands tailings water. These cultures were created by diluting a 10-ml sample of oil sands tailing into 90 ml of mineral salts medium that contained either NAS (100 mg/l) or TEX (1:50 dilution). The mineral salts medium was modified Bushnell-Haas medium. Successive transfers 1% v/v) of the enrichment culture into fresh NAS- to TEX-containing medium were on monthly basis and incubated at room temperature on a gyratory shaker (100 rpm). The viable cell number within each enrichment culture was estimated using the plate count technique.

**Experiment No. 1.** A measurement of $CO_2$ production was used to evaluate the ability of the enrichment cultures to mineralize components within both the NAS and TEX mixtures. Mineralization experiments were performed using 60-ml serum bottles containing 15 ml of growth medium. The growth medium consisted of sterilized mineral salts medium with NAS (100 mg/l) or TEX (1:20 and 1:50 dilutions) as the sole carbon source. Dissolve organic carbon analyses showed that 100 mg/l of NAS contained 60 mg C/l, while 1:20 and 1:50 dilutions of TEX contained 50 and 21 mg C/l, respectively. The serum bottles were

inoculated with 0.15 ml of either the NAS-degrading or the TEX-degrading enrichment culture, sealed with rubber stoppers, and incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 6-day intervals over 24 to 30 days, three inoculated bottles and one control (inoculated but lacking NAS or TEX) were acidified to pH <2 using 1 ml of 1M $H_2SO_4$ to convert all forms of inorganic carbon into $CO_2$. A 0.5 ml headspace sample from each bottle was analyzed for $CO_2$ content by gas chromatography. Mineralization of the organic substrate was first corrected for the amount of $CO_2$ in the control bottles, then expressed either as the total amount of $CO_2$ produced within the bottle or as the percentage of organic carbon converted to $CO_2$.

**Results of Experiment No. 1.** The mineralization studies showed that the NAS- and TEX-degrading enrichment culture was capable of mineralizing components within both the NAS and TEX mixtures. The percentage of organic carbon converted to $CO_2$ by the NAS-degrading culture was 48% (day 24) in the NAS bottles and 20% (day 20) in the TEX bottles. The percentage of organic carbon converted to $CO_2$ by the TEX-degrading culture was 34% (day 30) for the TEX bottles and 20% (day 25) for the NAS bottles.

**Experiment No. 2.** Mineralization of the four model naphthenic acid compounds was measured as the amount of $CO_2$ evolved from incubating solutions of the compounds dissolved in nutrient medium and inoculated with enrichment cultures of NAS-degrading microorganisms, TEX-degraders, or oil sands tailings pond water (TPW). Fifteen milliliters of 1 mM solutions of the compounds dissolved in mineral salts medium were placed in 60-ml serum bottles and inoculated (1% v/v) with the different sources of microbes then sealed with robber stoppers. Bottles were incubated at room temperature on a gyratory shaker (100 rpm). After 3, 6, 12, and 24 days, duplicate bottles were acidified and headspace $CO_2$ determined by GC. The level of $CO_2$ production was corrected for the amount of $CO_2$ within the control bottles and expressed as the percentage of organic substrate converted to $CO_2$.

**Results of Experiment No. 2.** The following results were obtained:.

Mineralization by day 24, % organic C converted to $CO_2$:

| Substrate | NAS-degraders | TEX-degraders | TPW |
|---|---|---|---|
| CCA | 41 | 56 | 57 |
| CPA | 45 | 57 | 58 |
| 2MCCA | 47 | 7 | 67 |
| 4PCCA | 6 | 24 | 24 |

**Experiment No. 3.** A 1% (v/v) inoculum of the NAS-degrading enrichment culture was placed in 125-ml Erlenmeyer flasks containing 50 ml of either NAS (30 mg/l) or TEX (1:50 dilution) in mineral salts medium. Control flasks received inoculum of heat-killed cells. The flasks were incubated at room temperature on a gyratory shaker (100 rpm). After an incubation period of 4, 8, and 16 days for NAS and 6, 12, and 24 days for TEX, the contents of two flasks and two control flasks were extracted for GC analysis. Samples were extracted and the carboxylic acids were derivatized to methyl esters prior to analysis.

Derivatized extracts were analyzed by GC with a capillary column and flame ionization detector.

**Results of Experiment No. 3.** Chromatographic analysis of solution from the control flasks revealed an unresolved series of many overlapping peaks that created a hump in the GC profile. When the mixture that was inoculated with NAS-enrichment culture, a reduction in the size of the hump was evident within 4 days, indicating that components within the naphthenic acid mixture were being degraded. Chromatographic analysis of the TEX samples revealed a similar hump of many overlapping peaks that appeared in the NAS GC profile. Biodegradation of TEX by the NAS-degrading culture did not result in a noticeable reduction in the size of the hump associated with TEX, despite evidence of mineralization of components within the mixture.

**Experiment No. 4.** A measurement of $CO_2$ production and $O_2$ utilization within sealed microcosms was used to monitor microbial activity in samples of TPW, and to determine the effect of nutrient addition (N and P) or carbon substrate addition (cyclohexane pentanoic acid (CPA), sodium salts of naphthenic acids (NAS), or tailings pond extracts of carboxylic acids (TEX)) on the level of microbial activity within TPW.

60 ml of TPW was placed into sterile 125-ml Erlenmeyer flasks, sealed with rubber stoppers in which a sampling port had been drilled and then sealed with clear silicone. Nutrients in the form of N and P were added. Carbon substrates (CPA, NAS or TEX) were added as a filter-sterilized solution to crate a final concentration of 60 mg organic carbon/l. All flasks were incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 80day intervals, 0.5 ml of headspace was sampled and analyzed for $CO_2$ and $O_2$ using GC. Following 5 weeks of incubation, the contents of the flasks containing CPA were extracted and analyzed using the procedure described for the GC analysis in experiment 3.

**Results of Experiment No. 4.** The addition of CPA to TPW resulted in increased microbial activity, as indicated by greater levels of $CO_2$ production and $O_2$ utilization when compared with TPW alone. Sterilized TPW demonstrated no $CO_2$ production or $O_2$ utilization. Even greater levels of microbial activity were evident when N and P were added in addition to CPA, indicating that mineralization could be enhanced by the addition of mineral nutrients. GC analysis of CPA in TPW microcosms after 35 d of incubation revealed that the concentration of CPA was below the level of detection in 2/3 microcosms and reduced 10-fold in the third microcosm. There was no detectable CPA in the three N and P-amended microcosms.

Similarly, NAS and TEX additions to microcosms increased microbial activity in TPW, although microbial activity was enhanced by the addition of N and P. Increases in both $CO_2$ evolution and $O_2$ utilization were seen.

**Conclusions.** This investigation showed that naphthenic acids, either as a commercial preparation of sodium salt (NAS) or natural extracts from oil sands tailing water (TEX) are capable of being utilized by natural assemblages of microorganisms. Addition of nitrogen and

| | |
|---|---|
| | phosphorus enhances the utilization of these substrates by the microbes. |
| **Source:** | Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oils sands tailings. |
| **Reliability:** | (2) Reliable with restrictions. The report was a well-documented study that meets basic scientific principles. |

# 4. Ecotoxicity

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

**Test Substance:** Naphthenic acids

**Method/Guideline:** Hart, et al. 1945; Doudoroff et al. 1951

**Year (guideline):** N/A

**Type (test type):** Static

**GLP:** No

**Year (study performed):** 1965

**Species:** zebra fish (*Brachydanio rerio*)

**Analytical Monitoring:** No

**Exposure Period:** 96 hours

**Statistical Method: (FT - ME)** Graphical interpolation for determining the LC50.

**Test Conditions: (FT - TC)**

- **Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**

Test containers were 2.5 gallon aquariums, each fitted with an air stone through which compressed air was bubbled to maintain a 5-9 ppm dissolved oxygen concentration in the dilution water. The aquariums were maintained at a temperature of 24 +/- 1 °C. Dilution water was synthetic soft water prepared with distilled water and ACS grade chemicals.

The lot of test fish displayed no visible disease. The average size was 3.2 cm total length. Before testing the fish were acclimated to the dilution water for 5 days. During the acclimation period they were fed *Daphnia* and white worms, but were not fed for 36 hours before or during the testing.

Test concentrations were prepared by direct addition of the test substance to the test chambers followed by mixing. Test concentrations were control, 7.5, 8.7, 10, 11.5, 13.5, 15.5, 18.0, 21.0, and 24.0 ppm naphthenic acids. After the test solutions were prepared, ten fish were placed in each test container. Controls were run in duplicate, while test levels were run singly. Mortality was evaluated at 24, 48, and 96 hours, and the criteria for death was a cessation of gill movement and failure to respond to mechanical stimulus.

Following the 96 hour test period the TLm (median tolerance limit) was determined from visual observation of the dose-response pattern. Where no exact TLm response resulted, the TLm was interpolated from a plot of the concentration and survival data on semi-log paper.

**Results: (FT - RS)** 96-hour TLm = 16.3 ppm

**Units/Value:**

The following dose-response data were provided:

| Concentration of Naphthenic acids, ppm | Number Tested | % Dead at 96 hours |
|---|---|---|
| 0 (control #1) | 10 | 0 |
| 0 (control #2) | 10 | 0 |
| 7.5 | 10 | 0 |
| 8.7 | 10 | 40 |

| | | |
|---|---|---|
| 10 | 10 | 20 |
| 11.5 | 10 | 0 |
| 13.5 | 10 | 20 |
| 15.5 | 10 | 30 |
| 18 | 10 | 80 |
| 21 | 10 | 100 |
| 24 | 10 | 100 |

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.** The article reported that pH and dissolved oxygen concentrations were taken during the test, but these data were not reported.

**Conclusion: (FT - CL)**

**Reliability: (FT - RL)** (2) Reliable with restrictions. The test was conducted under referenced test conditions current for the period in which the study was run. The report provided sufficient details for assessment.

**Source: (FT - RE)** Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus Raf. Notulae Naturae. No. 381:1-9.

Hart, W.B., P. Doudoroff, and J. Greenbank. 1945. The evaluation of the toxicity of the industrial wastes, chemicals and other substances to freshwater fishes – The Atlantic Refining Company, Philadelphia, PA. 315 pp.

Dourdoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galstoff, W.B. Hart, T. Patrick, E.R. Strong, E.W. Surber, and W.M. VanHorn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sew. and Ind. Wastes. 23(11):1380-1397.

**Other (source): (FT - SO)**

FT - Freetext
ME - Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

**Test Substance:** Naphthenic acid mixture (commercially available from Eastman Chemicals)

**Method/Guideline:** Peltier and Weber 1985

**Year (guideline):** 1985

**Type (test type):** static acute

**GLP:** not stated

**Year (study performed):**

**Species:** three-spine stickleback (*Gasterosteus aculeatus*)

**Analytical Monitoring:** no

**Exposure Period:** 96 hours

**Statistical Method: (FT - ME)**

**Test Conditions: (FT - TC)**

- **Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**

**Summary of Test Conditions**

| | |
|---|---|
| Organism age: | juvenile |
| Test Temperature: | 20 °C +/- 2 °C |
| Photoperiod: | 16 h light/8 h dark |
| Light intensity: | 10 – 50 micro-einsteins |
| Light quality: | wide spectrum fluorescent |
| Test container: | 5 gallon aquaria |
| Dilution water: | Carquinex Strait |
| Test Volume: | 15 liters |
| Animals per container: | 10 |
| Replicate containers: | 2 |
| Number of concentrations: | 6 (5 concentrations and a control) |
| Food: | none |
| Test duration: | 96 h |
| Test endpoint: | mortality |
| Salinity | 15 parts per thousand |
| Test pH: | ambient |
| Test article: | Martinez Refinery effluent (non-toxic) with added naphthenic acids |

Test solutions were prepared by creating a 1 percent solution using non-toxic effluent pH adjusted to 12 with sodium hydroxide. The stock solution was mixed overnight prior to use. The stock solution was used to spike non-toxic treated effluent to nominal naphthenic acid concentrations from 2.5 to 30 mg/l.

Test organisms were held at least seven days prior to testing in dilution water. During testing at 24-h intervals, the salinity, temperature, pH, and dissolved oxygen were measured in all control and test tanks. Survival data were taken at 24-h intervals and dead individuals were removed when observed.

**Results: (FT - RS)**

**Units/Value:** LC50 estimated to be in the range of 5 mg/l.

The following dose response data were reported:

| Concentration (mg/l) | % Survival |
|---|---|
| 0 (control) | 100 |
| 2.5 | 60 |
| 5 | 10 |
| 10 | 0 |
| 15 | 0 |
| 30 | 0 |

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**

Although an LC50 could have been calculated using contemporary methods, the author elected to estimate its value. The report stated that water chemistry data were collected but no data were reported.

**Conclusion: (FT - CL)**

**Reliability: (FT - RL)** (2) Reliable with restrictions. A statistically-defined LC50 was not calculated. Water chemistry data were not reported.

**Source: (FT - RE)** Dorn, P.B. 1992. Case Histories – The petroleum refining industry. In: Ford, D.L. (ed.). Water Quality Management Library, Volume 3, Toxicity Reduction Evaluation

and Control. Technomic Publishing Co., Lancaster, PA. pp 183 – 223.

Peltier, W.H., and C.I. Weber, eds. 1985. Method for measuring acute toxicity of effluents to freshwater and marine organisms, 3rd edition. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-85-014. 230 pp.

Stephan, C.E. 1977. Method for calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA. pp 65-84.

**Other (source): (FT - SO)**

FT - Freetext
ME - Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

## 4.9 ADDITIONAL REMARKS

**Memo:** Effect of naphthenic acids on survival of zebra fish (*Brachydanio rerio*) embryos

**Remark:** Zebra fish embryos were exposed for 48 hours to a range of naphthenic acids concentrations to determine the TLm (median tolerance limit) for embryo survival. Embryos were collected from a culture unit once they attained Stage 21 as designated by Hisaoka and Battle (1958). Ten embryos were exposed to each test solution and control in petri dishes holding 45 ml of the exposure solutions. Exposure solutions were prepared by diluting a stock solution of naphthenic acids (100 mg naphthenic acids in 50 ml acetone) with water. In addition to a control group, nine concentrations of naphthenic acids were prepared at 2.4, 3.2, 4.2, 6.5, 10, 15.5, 24, 32, and 42 ppm naphthenic acids. Mortality was assessed at 24 and 48 hours of exposure. The embryo was considered dead if it had an opaque appearance.

A TLm of 3.5 ppm was obtained by plotting the survival versus concentration on semilog paper and interpolating the 50% survival concentration. The following dose response was given:

| Test Concentration, ppm | Percent Dead |
|---|---|
| 0 (control) | 0 |
| 2.4 | 0 |
| 3.2 | 40 |
| 4.2 | 70 |
| 6.5 | 100 |
| 10 | 100 |
| 15.5 | 100 |
| 24 | 100 |
| 32 | 100 |
| 42 | 100 |

**Source:** Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus Raf. Notulae Naturae. No. 381:1-9.

Hisaoka, K.K., and H.I. Battle. 1958. The normal development stages of the zebra-fish, *Brachydanio rerio* (Hamilton-Buchanan). J. Morph. 102(2):311-327.

**Reliability:** (2) Reliable with restrictions. Although the test was conducted prior to the time of standardized test methods, the report provided sufficient information on the dose-response pattern for the test substance.

**Memo:** Effect of naphthenic acids on survival of bluegill (*Lepomis macrochirus*)

**Value:** 48-hour TLm = 5.6 mg/l naphthenic acids

**Remark:** The value was reported in a summarized journal article (Cairns et al., 1965) as originating in Cairns and Scheier (1962).

**Source:** Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus Raf. Notulae Naturae. No. 381:1-9. Acad. Nat. Sci. Philadelphia.

Cairns, J. Jr., and A. Scheier. 1962. The effect of temperature and hardness of water upon the toxicity of naphthenic acids to the common bluegill (Lepomis macrochirus Raf.) and the pond snail (Physa heterostropha Say). Notulae Naturae. No. 353: 111 pp. Acad. Nat. Sci. Philadelphia.

**Reliability:** (3) Not reliable. The endpoint was cited in the text of a journal article without details of the test.

**Memo:** Effect of naphthenic acids on survival of bluegill (*Lepomis macrochirus*)

**Value:** 96-hour LC50 = 0.0026 mg/l

**Remark:** Test chambers were 30x60x30 cm all-glass vessels. Dilution water was well water. Testing was performed at a temperature of 22 +/- 1°C under a 16-h light/8-h dark photoperiod.

The test included five concentrations of the test substance and a dilution water control. Each test level included 20 fish distributed 10 each to two replicate chambers per treatment.

Dissolved oxygen ranged from 4.3 to 8.1 mg/l, pH ranged from 7.4 to 8.0, and temperature ranged from 22 to 24 °C when measured daily during the test. Specific conductance between the test solutions remained constant at 550 (no units given) when measured at the beginning of the test.

The report stated that serial dilutions of the test product were created for testing, although no details were given as to how the serial dilutions or the original solution was created. The raw data indicated that concentrations were expressed as a percent, while the LC50 and confidence interval was reported as parts per million. There was no explanation how the values for percent were related to parts per million.

Critical details of testing procedures and animal culture were omitted from the report.

**Source:** Exxon Corporation. 1980. Aquatic bioassay testing of Exxon Corporation's experimental compounds (MRD 78-100). Report by Battelle Columbus Laboratories, Columbus, Ohio.

## 5. Acute Toxicity

### 5.1.1 ACUTE ORAL TOXICITY

| | |
|---|---|
| **Type:** | $LD_{50}$ |
| **Value:** | 5.88 (4.31-8.02) g/kg bw |
| **Species:** | Rat |
| **Strain:** | Wistar |
| **Sex:** | Male |
| **Number of Animals:** | 5 per dose level (7 dose levels) |
| **Vehicle:** | None – administered undiluted |
| **Year:** | 1979 |
| **GLP:** | Unable to determine |
| **Test Substance:** | MRD-79-10 (Raw naphthenic acid derived from kerosene ) |
| **Method** | Seven groups of 5 male rats were dosed at 1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg of body weights. Food and water were freely available except for the 16-20 hours prior to dosing.<br><br>The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology. |
| **Result:** | Deaths occurred at the four highest dose levels: 3.26, 4.64, 6.81, and 10 g/kg bw. 8/10 animals died at the two highest dose levels. Significant predeath toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died during the study included dilated hearts and gastrointestinal irregularities.<br><br>The $LD_{50}$ was determined to be 5.88 (4.31-8.02) g/kg bw |
| **Reliability:** | (1) Reliable without restrictions; appears to be comparable to a guideline study with adequate experimental details provided; although the investigators used male rats only, there is sufficient experimental detail to make a conclusion on the study's validity, and the results can be used to assess the potential acute toxicity of naphthenic acid. |

| | |
|---|---|
| **Source** | Exxon, Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, 1979. |
| **Type:** | $LD_{50}$ |
| **Value:** | 3.0 g/kg bw (fraction from crude kerosene acids)<br><br>5.2 g/kg bw (fraction from mixed crude oils) |
| **Species:** | Rat |
| **Strain:** | No information |
| **Sex:** | No information available |
| **Number of Animals:** | "Sufficient animals ...so the the LD50 dose could be computed by either the Weil or the Litchfield and Wilcoxon method" |
| **Vehicle:** | None – administered undiluted |
| **Year:** | 1955 |
| **GLP:** | Unable to determine |
| **Test Substance:** | 1) 7-93% Naphthenic acid fraction from crude kerosene acids<br><br>2) 65-69% Naphthenic acid fraction from mixed crude oils |
| **Method** | "The LD50 ..was determined in rats by use of screening test procedures similar to those of Smyth and Carpenter." (Smyth, H.F., and C.P. Carpenter. 1944. Place of the range finding test in the industrial toxicology laboratory. J. Indust. Hyg. & Tox. 26: 269. |
| **Result:** | Death appears to result from gastrointestinal disturbances, with the mortality peak occurring on the third to fourth day after administration. The animals exhibited anorexia, inanition, diarrhea, and asthenia.<br><br>The $LD_{50}$s were determined to be 3.0 g/kg bw (fraction from crude kerosene acids) and 5.2 g/kg bw (fraction from mixed crude oils) |
| **Reliability:** | (2) Reliable with restrictions; Although not a guideline or GLP study, and some of the experimental details are not available, the study does appear to be well-conducted, and cites that the investigators followed published methodologies for conducting a statistically valid LD50. The data are supportive of other acute toxicity studies reported by Exxon and Pennisi. |
| **Source** | Rockhold, W.T. 1955. The toxicity of naphthenic acids and their metal salts. Archs Ind Hlth 12, 477-482. |

| | |
|---|---|
| **Type:** | $LD_{50}$ |
| **Value:** | 3550 mg/kg bw |
| **Species:** | Mice |
| **Strain:** | White – no other information |
| **Sex:** | Male |
| **Number of Animals:** | No information available |
| **Vehicle:** | No information available |
| **Year:** | 1977 |
| **GLP:** | Unlikely |
| **Test Substance:** | Naphthenic Acid – no further description |
| **Method** | Not described |
| **Result:** | Oral administration resulted in 1) CNS depression without analgesia and no loss of corneal reflex, 2) corneal eye opacity, 3) dryness of mouth, 4) convulsions, 5) diarrhea, and 6) death due to respiratory arrest. |
| **Reliability:** | (4) Not assignable. This information is taken from a published, meeting abstract. The level of experimental details provided is not sufficient to verify the conclusions. |
| **Source** | Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181. |

| | |
|---|---|
| **Type:** | Acute Oral Toxicity Study (Not LD50) |
| **Value:** | Not applicable |
| **Species:** | Rat |
| **Strain:** | Wistar |
| **Sex:** | Male/Females |
| **Number of Animals:** | 10 Females/dose (3 doses, plus control)<br>10 Males/dose (1 dose, plus control) |
| **Vehicle:** | Aqueous solutions of naphthenic acids/Water |
| **Year:** | 2002 |

| | |
|---|---|
| **GLP:** | Unable to determine |
| **Test Substance:** | Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 55,080, 5508 or 550.0 mg/l naphthenic acids – derived from athabasca sands sands tailings. |
| **Method** | Female rats were given a single oral dose of naphthenic acids at 3, 30 or 300 mg/kg bw, while male rats received 300 mg/kg. Control animals were given tap water. All animals were monitored continuously for 12 hr after dosing, and thereafter daily. Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination.<br><br>Statistical analysis was performed by using a one-way ANOVA to compare means of female dose and control groups with respect to consumption, body weights, and organ weights. A pair wise multiple comparison test was then used in cases where statistical significance was reached. For the male dose and control groups, a Student's t-test was used to compare group means. Probability values of $p \leq 0.05$ was considered statistically significant. |
| **Result:** | The following effects were seen in the high dose groups:<br>• Decreased food consumption immediately following dosing.<br>• Lethargy and mild ataxia (2/10 females, 3/10 males)<br>• Statistically significant increase relative organ weights: ovaries, spleen in females- testes, heart in males<br>• 7/10 females and 6/10 males exhibiting eosinophilic pericholangitis<br>• 6/10 males and 2/10 females with brain hemorrhage.<br>The following effects were seen in the mid dose group:<br>• 7/10 females and 4/10 males with heart lesions. |
| **Reliability:** | (2) Reliable with restriction. The study is not an acute toxicity study as defined by OECD SIDS/HPV, however it appears to be well conducted and provides additional information regarding potential acute, non-lethal effects of naphthenic acids following oral exposure. |
| **Source** | Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355. |

### 5.1.2 ACUTE DERMAL TOXICITY (WITH IRRITATION)

| | |
|---|---|
| **Type:** | $LD_{50}$ |
| **Value:** | > 3.16 g/kg bw |
| **Species:** | Rabbit |

| | |
|---|---|
| **Strain:** | NZ White |
| **Sex:** | Male/Female |
| **Number of Animals:** | 2 per sex |
| **Vehicle:** | None – administered undiluted |
| **Year:** | 1979 |
| **GLP:** | Unable to determine |
| **Test Substance:** | MRD-79-10 (Raw naphthenic acid derived from kerosene ) |
| **Method** | 3.16 g/kg naphthenic acid was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil.<br><br>According to experimental protocol, no deaths occurred at the initial level, no addition animals were dosed. If one animal died, the experiment was to be repeated using 3 more groups of animals dosed at varying levels.<br><br>Following the skin wash, animals were observed for mortality and toxic effects at 2 hr and 4 hr, and once daily thereafter. Body weights were recorded pretest and at termination. Dermal irritation was recorded at 24 hr, 3, 7, 10 and 14 days.<br><br>The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology. |
| **Result:** | No deaths occurred at the 3.16 mg/kg dose level. Most of the animals (3/4) appeared normal during the first 2 to 4 hours of dosing, after which symptoms of toxicity were observed. 3 out of 4 animals (1 male, 2 female) showed signs of toxicity until day 12 or 13. During the first 5 days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces.<br><br>The $LD_{50}$ was determined to be greater than 3.16 g/kg bw<br><br>Redness and irritation scores were recorded at 24 hr, 3, 7, 10 and 14 days post-washing.<br><br>4 Hour occluded sites (DOT, OECD methods)<br>Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 1.69 and 1.3 respectively.<br>The initial response of the skin to the test material was slight, with little difference in response between intact or abraded sites. |

The material was judged to be moderately to severely irritating to the occluded skin.

Actual scores were:

**Erythema/Eschar Scores**

| Animal Number | 1 day | 3 day | 7 day | 10 day | 14 day |
|---|---|---|---|---|---|
| 1M | 2 | 2 | 4 | 4 | 1 |
| 2M | 1 | 2 | 4 | 4 | 1 |
| 3F | 2 | 4 | 4 | 4 | 0 |
| 4F | 2 | 3 | 4 | 4 | 0 |
| Note: All animals showed signs of scar formation after 14 days. | | | | | |

**Edema**

| Animal Number | 1 day | 3 day | 7 day | 10 day | 14 day |
|---|---|---|---|---|---|
| 1M | 3 | 2 | 2 | 2 | 1 |
| 2M | 2 | 3 | 2 | 2 | 0 |
| 3F | 3 | 3 | 2 | 2 | 0 |
| 4F | 3 | 3 | 2 | 2 | 0 |

**Reliability:** (1) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.

**Source** Exxon, Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, 1979.

### 5.2.1 EYE IRRITATION

**Type:** EYE IRRITATION

**Species:** Rabbit

**Strain:** NZ White

**Sex:** Male/Female

**Number of Animals:** 3 per sex

| | |
|---|---|
| **Concentration :** | None – administered undiluted |
| **Year:** | 1979 |
| **GLP:** | Unable to determine |
| **Test Substance:** | MRD-79-10 (Raw naphthenic acid derived from kerosene ) |
| **Method** | 0.1 ml naphthenic acid was placed into the conjunctival sac of eye of each of the six rabbits. The lids were held together briefly to insure adequate distribution. The untreated eye served as a control.<br><br>The rabbits were observed at 1 and 4 hours, and on days 1, 2, 3, 4, and day 7. If a positive score (any score for iritis or opacity, or a score of 2 or more for redness or chemosis) was noted on day 7, ocular reactions were scored on day 10. Likewise readings on day 14 were given if there was a positive reaction on day 10. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system. |
| **Result:** | The following is a summary of data taken from the report:<br>One animal had a positive corneal score that was noted on days 1 and 2. One animal had a positive iris score which was noted during hours 1 and 4. All animals exhibited positive conjunctival scores at some pint during the first three days of observation. By day 4, no animals showed positive scores.<br>abraded sites.<br><br>The material was judged to be an irritant. (According to Draize chart, 4 to 6 rabbits with positive scores observed at 24, 48 or 72 hours). In a later Exxon summary report, eye irritation was judged to be moderate (Exxon, 1980). |
| **Reliability:** | (1) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity. |
| **Source** | Exxon, Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, 1979. |

### 5.4 REPEATED DOSE TOXICITY

| | |
|---|---|
| **Type:** | Subchronic (90 Day) |
| **Species:** | Rat |
| **Sex:** | Females |
| **Strain:** | Wistar |
| **Route of administration:** | Oral |

| | |
|---|---|
| **Exposure period:** | 90 days |
| **Frequency of treatment:** | 1 dose/day (Mon. – Fri, 5 days/week) |
| **Doses/No. of animals:** | 0.6, 6 or 60 mg/kg bw (aqueous solutions of naphthenic acids); 12 animals per dose level |
| **Control group:** | Water – 7.0 ml tap water |
| **Year:** | 2002 |
| **GLP:** | Unable to determine |
| **Test Substance:** | Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 8549, 845.9 or 84.50 mg/l naphthenic acids derived from Athabasca sands sands tailings. |
| **Method:** | Female rats were administered naphthenic acid (orally) at doses of 0.6, 6, or 60 mg/kg/day, 5 days per week for 90 days. Control animals were given 7 ml tap water. All animals were monitored daily . Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Similarly, blood samples taken via cardiac puncture on day 91 were analyzed. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination.<br><br>Statistical analysis was performed by using a one-way ANOVA to compare group means for consumption, plasma biochemical/ hematological parameters , and organ weights, while a one-way repeated measure ANOVA was used to compare body weight trends. Probability values of $p \leq 0.05$ was considered statistically significant. |
| **Result:** | The following significant effects were seen in the high dose groups:<br>• Decreased food consumption immediately following dosing.<br>• Severe, clonic seizures lasting 20 sec (25%) of animals, observed after day 40 – after which all animals, except one that died, resumed normal activity.*<br>• Lower mean body weight throughout the exposure period.<br>• Increased relative organ weights: liver, kidney and brain<br>• Reduction in plasma cholesterol on days 45 and 91 (41 and 43%), Increase in amylase activity on day 45 and 91 (33 and 30%)<br>• Less pronounced differences in total protein concentration (increased) and albumin/globulin ratio (decreased)<br>• 5/12 rats with increased glycogen storage.<br><br>The following effects were seen in the mid-dose group:<br>• Severe, clonic seizures lasting 20 sec (17%) of animals, observed after day 40 – after which all animals except one that died, resumed normal activity.*<br>• 3/12 rats with increased glycogen accumulation<br><br>The following effects were seen in the low-dose group: |

| | |
|---|---|
| | • 2/12 rats with increased glycogen accumulation<br><br>*Note: Rats in the low-dose (8%) and control (17%) demonstrated milder episodes, characterized primarily by muscle twitching.<br><br>Dose-related changes in liver tissue with respect to glycogen accumulation. |
| **Reliability** | (2) Reliable with restriction. The study is not a typical subchronic toxicity study as defined by OECD SIDS/HPV, i.e., the study was conducted with female rats only and examined a limited number of organs. However, it is well-conducted and provides limited information regarding potential subchronic effects of naphthenic acids following oral exposure. |
| **Source:** | Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355. |
| **Type:** | Subchronic (30 Day) |
| **Species:** | Mice |
| **Sex:** | Male |
| **Strain:** | Wistar |
| **Route of administration:** | Oral |
| **Exposure period:** | 30days |
| **Frequency of treatment:** | Daily |
| **Doses/No. of animals:** | 1000 mg/kg bw (no information on number of animals per dose) |
| **Control group:** | No information available |
| **Year:** | 1977 |
| **GLP:** | Unlikely |
| **Test Substance:** | Naphthenic acid – no further information. |
| **Method:** | Male rats were given daily oral doses of 1000 mg/kg naphthenic acids. No other experimental details provided in abstract. |
| **Result:** | The following statements appeared in the abstract:<br>Repeated daily administration (30 days) of naphthenic acid at doses of 1000 mg/kg orally .. revealed a few cases of (1) CNS depression without analgesia and no loss of the corneal reflex (2) hematological changes, (3) weight loss leading eventually to death due to respiratory arrest, (4) gross morphological changes in the liver and stomach, and (5) histomorphological changes in a few selected organs. |
| **Reliability** | (4) Not assignable. This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline |

study, and the level of detail is insufficient to judge its validity.

**Source:**

Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181. [meeting abstract]

### 5.5 GENETIC TOXICITY IN VITRO

The following salts of naphthenic acid were tested using National Toxicology Program protocols and conducted in accordance with GLP's. Consequently they have ratings of (1), reliable without restriction:

| | Calcium Naphthenate | Sodium Naphthenate |
|---|---|---|
| **Salmonella Mutagenicity Test** | Negative | Negative |
| **Chromosome Aberration Test** | --- | Negative |
| **Sister Chromatid Exchange Test** | --- | Positive |

**Source:** NTP. 2003. http://ntp-server.niehs.nih.gov/htdocs/Overviews/GenProtocolsPg.html.

## 5.6 GENETIC TOXICITY IN VIVO

No data available.

## 5.7 CARCINOGENICITY

| | |
|---|---|
| **Species:** | Mice |
| **Sex:** | Female |
| **Strain:** | No information available |
| **Route of administration:** | Dermal |
| **Exposure period:** | 2 yr |
| **Frequency of treatment:** | 2 times/day |
| **Doses/No. of animals:** | 0.05 ml neat - 50 animals |
| **Control group:** | No information available |
| **Year:** | 1987 |
| **GLP:** | Unknown |
| **Test Substance:** | Calcium naphthenate |
| **Method:** | Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)" |
| **Result:** | The following statements appeared in the abstract:<br><br>Clinical observations included mild irritation, hair loss, shiny patches on the skin, and flaking skin surfaces. These progressed to moderate irritation (observed with sores and scabs on the treated site), or severe irritation caused by large sores or visible ulcers. In the negative control group, no cutaneous tumors developed at or distant to treated sites. Twelve epidermal and one dermal tumor at the treated sites were observed in eight mice that were exposed to the test material. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present. |
| **Reliability** | (4) Not assignable. This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary. |
| **Source:** | U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). http://www.epa.gov/opptintr/chemtest/naphthst.htm. |

## 5.8 EFFECTS ON REPRODUCTION

| | |
|---|---|
| **Type:** | One Generation Reproduction |
| **Species:** | Rabbit |
| **Sex:** | Male (10)/Female (2) |
| **Strain:** | No information available |
| **Route of administration:** | Dermal |
| **Frequency of treatment:** | 6 hr/day, 5 d/wk, 10 weeks |
| **Doses/No. of animals:** | 2 ml (neat) – 10 male (2 female animals not treated) |
| **Control group:** | No information available |
| **Method:** | 10 week exposure of males prior to mating |
| **Year:** | 1984 |
| **GLP:** | Unknown |
| **Test substance:** | Calcium naphthenate |
| **Method:** | Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)" |
| **Result:** | The following statements appeared in the available summary:<br><br>There were no systemic toxicity, application site toxicity, or statistically significant changes in body weights observed in the test animals during the 10 week exposure period or the 12 week post-exposure period. In the male animals, there were no significant changes in the testes weights. In the females, there were no significant differences in the number of implantations, or in pre-and post-implantation losses. In addition, there were no differences in viable fetuses to those females that were mated with exposed males compared to those mated with unexposed males. The study also reported that there were no macroscopic or microscopic pathological findings in the male reproductive tract. |
| **Reliability:** | (4) Not assignable. This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary. |
| **Source:** | U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). http://www.epa.gov/opptintr/chemtest/naphthst.htm. |

## 5.9 DEVELOPMENTAL TOXICITY

| | |
|---|---|
| **Species:** | Rat |
| **Sex:** | Female |
| **Strain:** | Wistar |
| **Route of administration:** | Oral |
| **Dose:** | 0.6, 6 or 60 mg/kg bw |
| **Exposure period:** | "Pre-breeding, breeding and gestation" - no other details provided |
| **Frequency of treatment:** | Daily |
| **Year:** | 2002 |
| **GLP:** | Unknown |
| **Test Substance:** | Naphthenic acid isolated from Athabasca oil sands tailings. |
| **Method:** | Oral doses of 60 mg/kg/day were given to female rats during pre-breeding, breeding and gestation. |
| **Result:** | The following description was given:<br><br>Reproductive toxicity testing demonstrated dramatic effects on female fertility at an oral dosage of 60 mg/kg/day during pre-breeding, breeding and gestation. While control and low dose (6 mg/kg/day) animals achieved 93 and 100% reproductive success, respectively, only 7% of females dosed at 60 mg/kg/d successfully bore a litter. Total cholesterol of the latter group was 30% lower than controls. Mating and ovulation were comparable amongst control and dose groups, while fetal malformations were not apparent in any offspring. Results suggest that the dose-related infertility may be associated with poor embryonic implantation, an effect that might be secondary to depressed sex hormone production requiring cholesterol as a precursor. |
| **Reliability:** | (4) Not assignable. This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline study, and the level of detail is insufficient to judge. However, it may be useful in establishing dose levels for a more in-depth study. |
| **Source:** | Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002b. Mammalian toxicity of naphthenic acids derived from the Athabasca Oil Sands (AOS). Toxicologist 66(1-S): 64-5. [meeting abstract] |

# 1. General Information

**ID** 12002-85-3

**Date** December 22, 2005

**201-16126B**



## 1.0 SUBSTANCE INFORMATION

| | | |
|---|---|---|
| **Generic Name** | : | Zinc naphthenate |
| **Chemical Name** | : | Naphthenic acids, zinc salts |
| **CAS Registry No.** | : | 12001-85-3 |
| **Component CAS Nos.** | : | |
| **EINECS No.** | : | |
| **Structural Formula** | : | $Zn(MRCO_2)(NRCO_2)$<br><br>Where,<br>R = alkyl group with a chain length of 0 to 10 carbon atoms,<br>M & N are typically one or two fused rings (usually cyclopentane but occasionally cyclohexane or heptane rings) that may contain one or more alkyl substitutions. The total number of carbon atoms in M or N ranges from about 9 to 25. In some cases, no fused ring is present and M or N may be straight-chain or multiple branched carbon/hydrogen/oxygen molecules. |
| **Additional description** | : | This compound is the reaction product of zinc oxide and naphthenic acids, a petroleum refining by-product. Depending on the source of naphthenic acid, this compound may also contain 5 –20% paraffinic hydrocarbons which have a similar distillation range to the carboxylic acids. They cannot be removed by standard chemical processing and are not considered to be impurities, but rather legitimate components of naphthenic acid.<br><br>Zinc naphthenate may be a viscous liquid containing 8-10% zinc or a solid containing 16% zinc (EPA, 1992). |
| **Molecular Weight** | : | Ranges from approximately 381 to 813 |
| **Synonyms and Tradenames** | : | Fungitrol |
| **References** | : | EPA (1992). Drinking water toxicity profiles. U.S. Environmental Protection Agency. Report prepared for Army Medical Research and Development Command, Fort Detrick, Maryland. NTIS No. PB93122406. [Subsequently referenced as EPA, (1992)] EPA (1981). Chemical Hazard Information Profile - Draft Report. Cobalt Naphthenate, CAS No. 61789-51-3. U.S. Environmental Protection Agency, Office of Toxic Substances. 8 p. [Subsequently referenced as EPA, (1981)] |

## 2.1 MELTING POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | °C |
| **Decomposition** | : | at °C |
| **Sublimation** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** The pure form of zinc naphthenate is a cold flowing solid at room temperature. Because this substance is a mixture of many of different compounds, a distinct melting point is not expected. The melting point is the result of the transition from a highly ordered crystal form of a compound to the disordered liquid form. Zinc naphthenate is not expected to have a distinct melting point because it is highly disordered as a solid due to its unique chemical composition. |
| **Reliability** | : | |
| **Reference** | : | |

## 2.2 BOILING POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | ASTM D86-82 |
| **Value** | : | 116°C initial boiling point (pressure not specified) |
| **Decomposition** | : | Yes at 255°C |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (purity = 97%; 14.3% Zn) |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Test material was a very viscous liquid (i.e., light brown paste) |
| **Reliability** | : | (1) Reliable without restrictions. |
| **Reference** | : | Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P. |

## 2.3 DENSITY

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | ASTM D1475-60 (reapproved 1980) |
| **Value** | : | 1.118 g/ml at 20°C |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (purity = 97%; 14.3% Zn) |

| | | |
|---|---|---|
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Test material was a very viscous liquid (i.e., light brown paste) |
| **Reliability** | : | (1) Reliable without restrictions. |
| **Reference** | | Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P. |

## 2.4 VAPOR PRESSURE

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | <0.1 mm Hg (temperature not specified) |
| **Decomposition** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Mixture of 84% zinc naphthenate (14.5% Zn) and 16% petroleum hydrocarbon oil (CAS No. 64742-52-5) |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | Product MSDS, Sheperd Chemical Co. |

## 2.5 PARTITION COEFFICIENT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Partition coefficient** | : | |
| **Log Pow** | : | 1.10 at 20 °C |
| **pH value** | : | |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (purity = 97%; 14.7% Zn) |
| **Method** | : | |
| **Method detail** | : | Zinc as metal content in octanol was measured using ASTM method D2373-85. Zinc in water was measured by atomic absorption spectroscopy according to ASTM method E885-88. |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | (2) Reliable with restrictions. Test was not conducted at different pH values or in buffered water. |
| **Reference** | : | Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P. |

## 2.6.1 SOLUBILITY IN WATER

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | Water Solubility ASTM Method D2373-85 |
| **Value** | : | 80 mg/L at 20°C |
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |

| | | |
|---|---|---|
| **PKa** | : | at °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (purity = 97%; 14.7% Zn) |
| **Deg. products CAS#** | : | |
| **Method** | : | Flask Method conducted in accordance with reference (3) of Guideline 63-8(d) 40 CFR Part 158. |
| **Method detail** | : | Zinc in water was measured by atomic absorption spectroscopy according to ASTM method E885-88 |
| **Result** | : | |
| **Remark** | : | Test material was a very viscous liquid (i.e., light brown paste) |
| **Reliability** | : | (1) Reliable without restrictions. |
| **Reference** | : | Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P |

## 2.7 FLASH POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | >200 °C |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Mixture of 84% zinc naphthenate (14.5% Zn) and 16% petroleum hydrocarbon oil (CAS No. 64742-52-5) |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | Product MSDS, Sheperd Chemical Co. |

### 3.1.1 PHOTODEGRADATION

| | | |
|---|---|---|
| **Type** | | |
| **Guideline/method** | : | |
| **Light source** | : | |
| **Light spectrum** | : | |
| **Relative intensity** | : | based on |
| **Spectrum of substance** | : | lambda (max, >295nm) : |
| | | epsilon (max) : |
| | | epsilon (295) : |
| **Conc. of substance** | : | at °C |
| **DIRECT PHOTOLYSIS** | | |
| **Halflife (t1/2)** | : | |
| **Degradation** | : | % after |
| **Quantum yield** | : | |
| **INDIRECT PHOTOLYSIS** | | |
| **Sensitizer** | : | |
| **Conc. of sensitizer** | : | |
| **Rate constant** | : | |
| **Degradation** | : | |
| **Deg. product** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Deg. products CAS#** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

### 3.1.2 DISSOCIATION

| | | |
|---|---|---|
| **Type** | : | Dissociation constant determination |
| **Guideline/method** | : | OECD 112 |
| **pKa** | : | 7.31 and 9.18 at 20°C |
| **Year** | : | 2002 |
| **GLP** | : | Yes |
| **Test substance** | : | Zinc naphthenate (54458-2), lot number 20131MI, received from Aldrich Chemical Company. Clear gold liquid, purity not reported. |
| **Approx. water solubility** | : | 500 mg/L as determined visually in preliminary study |
| **Method** | : | OECD Guideline 112, Dissociation Constants in Water |
| **Method detail** | : | Three replicate samples of zinc naphthenate were prepared at a nominal concentration of 250 mg/L by dissolving 0.0250 grams of test substance in 100 mL of degassed water (ASTM Type II). Each sample was titrated against 0.005 N sodium hydroxide while maintained at a test temperature of 20±1°C. At least 10 incremental additions were made before the equivalence points and the titration was carried past the final equivalence point. Values of pK were calculated for a minimum of 10 points on the titration curve. Phosphoric acid and 4-nitrophenol were used as reference substances. |
| **Result** | : | Mean (N = 3) pKa values were 7.31 (SD = 0.0131) and 9.18 (SD= 0.0466) at 20°C |
| **Remark** | : | The results indicate that dissociation of the test substance will occur at environmentally-relevant pH values (approximately neutral) and at |

physiologically-relevant pH values (approximately 1.2).
**Supporting data for dissociation products:**
**Acid:** Naphthenic acids exist as weak acids, with most pKa values being reported at about 5. At low pHs, they exist in their undissociated form and tend to partition onto solids. At high pHs, they exist in their dissociated form and become more mobile (Appendix IIA)

**Reliability** : [1] Reliable without restriction.
**Reference** : Lezotte, F.J. and W.B. Nixon, 2002. Determination of the dissociation constant of naphthenic acids, zinc salts, Wildlife International, Ltd. Study No. 534C-121, conducted for the Metal Carboxylates Coalition.

### 3.2.1 MONITORING DATA

**Type of measurement** :
**Media** :
**Concentration** : mg/l
**Substance measured** :
**Method** :
**Method detail** :
**Result** :
**Remark** :
**Reliability** :
**Reference** :

### 3.3.1 TRANSPORT (FUGACITY)

**Type** :
**Media** :
**Air** : % (Fugacity Model Level I)
**Water** : % (Fugacity Model Level I)
**Soil** : % (Fugacity Model Level I)
**Biota** : % (Fugacity Model Level II/III)
**Soil** : % (Fugacity Model Level II/III)
**Year** :
**Test substance** :
**Method** :
**Method detail** :
**Result** :
**Remark** :
**Reliability** :
**Reference** :

### 3.5 BIODEGRADATION

**Type** :
**Guideline/method** :
**Inoculum** :
**Concentration** : related to
related to
**Contact time** :
**Degradation** : (±) % after day(s)
**Result** :
**Kinetic of test subst.** : % (specify time and % degradation)
%
%
%

| | | |
|---|---|---|
| | | % |
| **Control substance** | : | |
| **Kinetic** | : | % |
| | | % |
| **Deg. product** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Deg. products CAS#** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** Commercial mixtures of the sodium salts of naphthenic acids were shown to degrade and mineralize to $CO_2$ when inoculated with microbial populations indigenous to oil sands tailings. Approximately 50% of the organic carbon was converted to $CO_2$ over a 24-d period. Three of four model naphthenic acid compounds were also degraded by the enrichment cultures, with approximately 40-50% of the organic carbon converted to $CO_2$ over a 24-d period. Additional studies by Clemente et al. (2004) monitored the concentration and composition of naphthenic acids in aerobic biodegradation studies using sodium salts of naphthenic acids. Within 10 days of incubation with enrichment cultures on naphthenic acid-degraders, naphthenic acids concentration dropped from about 100 to <10 mg/L, accompanied by release of about 60% of the carbon as $CO_2$. GC/MS results indicated that the lower molecular weight acids (n = 5-13) were degraded more readily than high molecular weight acids. Clemente, J.S., M.D. Mackinnon, and P.M. Fedorak, 2004. Aerobic biodegradation of two commercial naphthenic acids preparations, Environ. Sci. Technol. 38:1009 – 1016. |
| **Reliability** | : | |
| **Reference** | : | |

## 3.7 BIOCONCENTRATION

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Exposure period** | : | at °C |
| **Concentration** | : | |
| **BCF** | : | |
| **Elimination** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

## 4.1 ACUTE TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | Static renewal |
| **Guideline/method** | : | FIFRA Guideline 72-1 |
| **Species** | : | Bluegill sunfish (*Lepomis macrochirus*) |
| **Exposure period** | : | 96 hr |
| **NOEC** | : | 1.0 mg a.i./L |
| **LC0** | : | |
| **LC50** | : | 1.5 mg a.i./L (1.1 – 2.0 mg a.i./L) |
| **LC100** | : | |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | Yes |
| **Year** | : | 1992 |
| **GLP** | : | Yes |
| **Test substance** | : | Zinc naphthenate, Lot # 24044-P, 98.9% active ingredient. Light brown viscous liquid |
| **Method** | : | FIFRA Guideline 72-1, Acute toxicity test for freshwater fish |
| **Method detail** | : | The test material was prepared in acetone. Ten fish per test concentration (5 per replicate test vessel, 0.15 grams of biomass per liter) were exposed under static conditions to five concentrations of the test material, control, and solvent control (0.5 mL acetone/L) in soft reconstituted water (hardness 38 mg/L as $CaCO_3$, pH 7.5) at a temperature of 19 - 21ºC. After 48 hours of exposure, all surviving fish were transferred to freshly prepared test solutions. This technique was used to maintain dissolved oxygen concentrations at acceptable levels. |
| **Result** | : | The mean measured concentrations averaged 94% of the nominal concentrations and were 5.0, 3.1, 1.7, 1.0 and 0.54 mg a.i./L. Complete mortality was observed at 96 hours at the two highest test concentrations. The 96-h LC50 was calculated to be 1.5 mg a.i/L (1.1 – 2.0 mg a.i./L). The NOEC was determined to be 1.0 mg a.i./L based upon sublethal effects (partial loss of equilibrium) seen in surviving fish exposed to 1.7 mg a.i./L. |
| **Remark** | : | **Supporting data for dissociation products:** **Acid:** Data in the U.S. EPA ECOTOX database from three references indicate an 96-h LC50 range for naphthenic acids of 5.6 – 7.1 mg/L for bluegill. The 96-h LC50 for another fish species, the zebra fish (*Danio rerio*), is reported as 16.3 mg/L for naphthenic acids. (U.S. Environmental Protection Agency. 2005. ECOTOX Database System. http://www.epa.gov/ecotox). |
| **Reliability** | : | [1] Reliable without restriction |
| **Reference** | : | Collins, M.K., 1992. Zinc Naphthenate – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) under Static Renewal Conditions. Springborn Laboratories, Inc. final report #92-3-4160, submitted to The Naphthenate Council c/o Mooney Chemicals, Inc., Cleveland, Ohio. |

| | | |
|---|---|---|
| **Type** | : | Static |
| **Guideline/method** | : | FIFRA Guideline 72-1 |
| **Species** | : | Rainbow trout (*Oncorhynchus mykiss*) |
| **Exposure period** | : | 96 hr |
| **NOEC** | : | 0.39 mg a.i./L |
| **LC0** | : | |
| **LC50** | : | 1.1 mg a.i./L (0.66 – 1.8 mg a.i./L) |
| **LC100** | : | |
| **Other** | : | |

| | | |
|---|---|---|
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | Yes |
| **Year** | : | 1992 |
| **GLP** | : | Yes |
| **Test substance** | : | Zinc naphthenate, Lot # 24044-P, 98.9% active ingredient. Light brown viscous liquid |
| **Method** | : | FIFRA Guideline 72-1, Acute toxicity test for freshwater fish |
| **Method detail** | : | The test material was prepared in acetone. Ten fish per test concentration (5 per replicate test vessel, 0.21 grams of biomass per liter) were exposed under static conditions to five concentrations of the test material, control, and solvent control (0.5 mL acetone/L) in soft reconstituted water (hardness 38 mg/L as $CaCO_3$, pH 7.4) at a temperature of 12 - 13ºC. |
| **Result** | : | The mean measured concentrations averaged 102% of the nominal concentrations and were 3.2, 1.8, 1.1, 0.66 and 0.39 mg a.i./L. Complete mortality was observed at 96 hours at the two highest test concentrations, with 50% mortality at the middle concentration and 0% mortality at the two lowest test concentrations. The 96-h LC50 was estimated by nonlinear interpolation to be 1.1 mg a.i/L (0.66 – 1.8 mg a.i./L). The NOEC was determined to be 0.39 mg a.i./L based upon sublethal effects (darkened pigmentation and partial loss of equilibrium) seen in several fish at the next highest test concentration. |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** Data in the U.S. EPA ECOTOX database from three references indicate an 96-h LC50 range for naphthenic acids of 5.6 – 7.1 mg/L for bluegill. The 96-h LC50 for another fish species, the zebra fish (*Danio rerio*), is reported as 16.3 mg/L for naphthenic acids. (U.S. Environmental Protection Agency. 2005. ECOTOX Database System. http://www.epa.gov/ecotox). |
| **Reliability** | : | [1] Reliable without restriction |
| **Reference** | : | Collins, M.K., 1992. Zinc Naphthenate – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) under Static Conditions. Springborn Laboratories, Inc. final report #92-3-4154, submitted to The Naphthenate Council c/o Mooney Chemicals, Inc., Cleveland, Ohio. |

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Type** | : | Static |
| **Guideline/method** | : | FIFRA Guideline 72-2 |
| **Species** | : | *Daphnia magna* |
| **Exposure period** | : | 48 hr |
| **NOEC** | : | |
| **EC0** | : | |
| **EC50** | : | 4.6 mg a.i./L (2.6 – 8.2 mg a.i/L) |
| **EC100** | : | |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | Yes |
| **Year** | : | 1992 |
| **GLP** | : | Yes |
| **Test substance** | : | Zinc naphthenate, Lot # 24044-P, 98.9% active ingredient. Light brown viscous liquid |
| **Method** | : | FIFRA Guideline 72-2, Acute toxicity test for freshwater aquatic |

| | | |
|---|---|---|
| | | invertebrates |
| **Method detail** | : | The test material was prepared in acetone. Twenty daphnids (≤ 24 h old) per test concentration (5 per replicate test vessel) were exposed under static conditions to six concentrations of the test material, control, and solvent control (0.5 mL acetone/L) in fortified well water (hardness 170 mg/L as $CaCO_3$, pH 8.1) at a temperature of 20 – 21ºC. |
| **Result** | : | The mean measured concentrations averaged 71% of the nominal concentrations and were 35, 20, 14, 8.2, 4.6 and 2.6 mg a.i./L. Complete immobilization was observed at 48 hours at the four highest test concentrations, with 50% immobilization at the 4.6 mg/L concentration and 0% immobilization at the lowest test concentration. The 48-h EC50 was estimated by nonlinear interpolation to be 4.6 mg a.i/L (2.6 – 8.2 mg a.i./L). The NOEC was determined to be 2.6 mg a.i./L (no immobiliztaion or sublethal effects). |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** A 96-h LC50 of 4.8 mg/L for calcium naphthenate has been reported for the marine copepod, *Nitocra spinipes*. (Bengtsson, B.E. and M. Tarkpea. 1983. The acute aquatic toxicity of some substances carried by ships. Mar. Pollut. Bull. 14:213-214). The zooplankton species Nephargoides maeoticus tolerated naphthenic acids concentrations up to only 0.15 mg/L (Dokholyan and Magomedov, 1984, cited in Clemente, J.S. and P.M. Fedorak, 2005, A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere 60:585-600). |
| **Reliability** | : | [1] Reliable without restriction. |
| **Reference** | : | Collins, M.K., 1992. Zinc Naphthenate – Acute Toxicity to Daphnids (*Daphnia magna*) under Static Conditions. Springborn Laboratories, Inc. final report #92-13-4089, submitted to The Naphthenate Council c/o Mooney Chemicals, Inc., Cleveland, Ohio. |

### 4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Endpoint** | : | |
| **Exposure period** | : | |
| **NOEC** | : | |
| **LOEC** | : | |
| **EC0** | : | |
| **EC10** | : | |
| **EC50** | : | |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** The toxicity of naphthenic acids to populations of the freshwater diatom, *Navicula seminulum*, has been measured. The 96-h EC50 for growth ranged from 26.0 – 80.5 mg/L (Academy of Natural Sciences. 1960. |

Cited in the EPA ECOTOX Database 2005. http://www.epa.gov/ecotox).

**Reliability** :

**Reference** :

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

| | | |
|---|---|---|
| **In vtro/in vivo** | : | |
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Number of animals** | : | |
| **Males** | : | |
| **Females** | : | |
| **Doses** | : | |
| **Males** | : | |
| **Females** | : | |
| **Vehicle** | : | |
| **Route of administration** | : | |
| **Exposure time** | : | |
| **Product type guidance** | : | |
| **Decision on results on acute tox. tests** | : | |
| **Adverse effects on prolonged exposure** | : | |
| **Half-lives** | : | 1st.:<br>2nd.:<br>3rd.: |
| **Toxic behavior** | : | |
| **Deg. product** | : | |
| **Deg. products CAS#** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

## 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | Limit Test |
| **Guideline/Method** | : | |
| **Species** | : | Rat (albino) |
| **Strain** | : | Sherman-Wistar |
| **Sex** | : | Male and female |
| **Number of animals** | : | 5 of each sex |
| **Vehicle** | : | None |
| **Doses** | : | Single dose of 5.0 g/kg given to all animals |
| **LD50** | : | > 5.0 g/kg |
| **Year** | : | 1980 |
| **GLP** | : | |
| **Test substance** | : | Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4) |
| **Method** | : | Described as similar to that in Federal Hazardous Substances Act regulations in 16 CFR 1500.3. |
| **Method detail** | : | One group of ten (5 male and 5 female) albino rats was used. Rats |

weighed between 200 and 300 grams each. Rats were deprived of food, but not water, overnight before dosing. Animals were dosed by direct administration into the stomach by means of a syringe and dosing needle. Following administration, the animals were allowed food and water *ad libitum* for the 14 day observation period during which rats were observed for signs of toxicity.

**Result** : There were no mortalities. Shortly after dosing, the animals were slightly letharegic and ruffled. They appeared normal after 24 hours. Gross pathological examination revealed nothing remarkable.

**Remark** : **Supporting data for dissociation products:**
**Acid:** Other data for rats includes an LD50 of 3.0 g/kg bw for naphthenic acid fraction from crude kerosene acids and 5.2 g/kg bw for naphthenic acid fraction from mixed crude oils (Rockhold, 1955, as cited in Appendix A of Appendix II). An oral acute toxicity test with a mixture of naphthenic acids isolated from Athabasca oil sands produced appetite suppression, hepatoxicity and cardiovascular effects with a single dose of 300 mg/kg. (Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355).
**Metal:** Acute oral toxicity in rodents exposed to zinc is low, and the level at which zinc produces no adverse effect in rats is approximately 160 mg/kg body weight (WHO, 2001, Environmental Health Criteria 221, Zinc). Of the compounds zinc nitrate, zinc sulfate, zinc chloride and zinc acetate, zinc acetate was the most toxic, with oral LD50 values of 237 mg Zn/kg bw (rat) and 86 mg Zn/kg bw (mouse). The LD50 for zinc chloride in an oral exposure was reported to be 528 mg Zn/kg bw in rats and 605 mg Zn/kg bw in mice (ATSDR, 1994, Toxicological Profile for Zinc).

**Reliability** : [2] Reliable with restrictions. Basic data given: comparable to guidelines.

**Reference** : Biosearch, Inc. (1980). Fungitrol Zinc 8% Toxicological Studies. Project number 80-2171A. Submitted to Tenneco Chemicals. [Available from the National Technical Information Service in microfiche OTS05151131, „Eight toxicological studies of naphthenic acids, zinc salts with attachments and cover letter dated 072187"][Subsequently referenced as Biosearch (1980)]

**Type** : Limit test
**Guideline/Method** : Oral Toxicity Single Dose, EPA 40 CFR 163.81-1 (Proposed)
**Species** : Rat
**Strain** : Sprague-Dawley
**Sex** : Five males and five females, weighing 200 – 300 grams each
**Number of animals** : 10
**Vehicle** :
**Doses** : Single dose of 5 g/kg administered to all animals
**LD50** : > 5 g/kg
**Year** : 1985
**GLP** : No
**Test substance** : 2% zinc naphthenate, in mineral spirits solvent. Sample density 0.82 g/mL
**Method** : Oral Toxicity Single Dose, EPA 40 CFR 163.81-1 (Proposed)
**Method detail** : Food (but not water) withheld 24 hours prior to dosing. Following dosing by gavage, food and water allowed *ad libitum*. Animals observed twice daily for 14 days, weight recorded after 7 and 14 days. All animals autopsied.

**Result** : Lethargy, piloerection and nasal discharge were observed in some animals following intubation. 1/5 females and 0/5 males died (death at 30 hours following intubation). All surviving animals appeared normal at 48 hours and no abnormal behavioral or physical symptoms were observed during the remainder of the observation period. Hemhorragic lungs, dark kidneys and pale spleen in the dead animal; all other animals had normal tissues and organs at autopsy.

| | | |
|---|---|---|
| **Remark** | : | |
| **Reliability** | : | [2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance. |
| **Reference** | : | Hoster, S., 1985. Acute Toxicology – Oral, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc. |
| **Type** | : | Oral LD50 |
| **Guideline/Method** | : | |
| **Species** | : | Rat |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | Stoddard-type solvent |
| **Doses** | : | |
| **LD50** | : | > 6.0 g/kg |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Zinc naphthenate containing 8.0% zinc |
| **Method** | : | Smyth & Carpenter (1944) |
| **Method detail** | : | Dosing by gavage |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | [4] Not reliable. Documentation insufficient for assessment. |
| **Reference** | : | Rockhold, W.T. 1955. Toxicity of naphthenic acids and their metal salts. A.M.A. Arch. Indust. Health. 12: 477-482. |

### 5.1.2 ACUTE INHALATION TOXICITY

| | | |
|---|---|---|
| **Type** | : | Limit test |
| **Guideline/method** | : | |
| **Species** | : | Rat (albino) |
| **Strain** | : | |
| **Sex** | : | Male and female |
| **Number of animals** | : | 5 of each sex |
| **Vehicle** | : | Mineral spirits |
| **Concentrations** | : | A single concentration of 11.6 mg/L was administered to all animals |
| **Exposure time** | : | 4 hr |
| **LC50** | : | >11.6 mg/L (for a 50% w/v suspension in mineral spirits) |
| **Year** | : | 1980 |
| **GLP** | : | Yes (per EPA's proposed GLP regulations at the time) |
| **Test substance** | : | Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4) |
| **Method** | : | Similar to that proposed in 40 CFR 163.81-3 (August 22, 1978). |
| **Method detail** | : | Animals were exposed to an aerosol of the test material inside a 260 liter plexiglass exposure chamber for four hours (flow rate of 20 L per minute). Following the exposure period, animals were returned to their cages and observed for a 14-d period. Signs of toxicity and mortalities were noted. The aerosol was generated by a six jet Collision nebulizer. Particle size of the aerosol was determined using an Andersen Sampler cascade impactor. The mass median diameter of particles was 0.54 μm, within the respirable range. The concentration of particles was 0.42 mg/L. |
| **Result** | : | There were no mortalites of exposed animals. Animals appeared depressed and ruffled within 18 to 24 hours after exposure, but returned to normal after 48 hours. Gross pathological examination revealed nothing remarkable. |
| **Remark** | : | |

| | | |
|---|---|---|
| **Reliability** | : | [2] Reliable with restrictions. Basic data given: comparable to guidelines. |
| **Reference** | : | Biosearch, Inc. (1980). |
| | | |
| **Type** | : | Limit test |
| **Guideline/method** | : | Inhalation toxicity – EPA (40 CFR 163.81-3) |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male and female, weighing 200 – 300 grams each |
| **Number of animals** | : | 5 of each sex for the exposure, 5 of each sex for the control |
| **Vehicle** | : | Mineral spirits |
| **Concentrations** | : | A single concentration (25.2 mg/L nominal, 0.72 mg/L assayed) was administered to all animals. |
| **Exposure time** | : | 4 hr |
| **LC50** | : | |
| **Year** | : | 1985 |
| **GLP** | : | No |
| **Test substance** | : | 2% zinc naphthenate in mineral spirits solvent |
| **Method** | : | Inhalation toxicity - EPA (40 CFR 163.81-3 ) |
| **Method detail** | : | Animals were exposed to an aerosol of the test material inside a 392 liter plexiglass exposure chamber for four hours (flow rate 20 L/min.). Sample (1000 g) was sprayed into the chamber with a Burgess Thermo Model F-982. Sample was sprayed for 15 seconds at 5 minute intervals for the first 15 minutes and then 5 seconds at 5 minute intervals for the remaining time. Following the exposure period, animals were returned to their cages and observed twice daily for a 14-d period. Signs of toxicity and mortalities were noted, and weights taken at 2,3,4 and 7 days. A group of 10 rats was held for a two week observation period under the same conditions. Particle size of the aerosol was determined using an Andersen Sampler, with 87-88% of the particles 9-10 μm or larger. |
| **Result** | : | There were no mortalites of exposed animals. Animals appeared normal at the end of the exposure period and for the duration of the observation period. Autopsies indicated one exposed animal and one control animal with hypervacuolization of the center of the right kidney; all other tissues and organs appeared normal. |
| **Remark** | : | |
| **Reliability** | : | [2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance. |
| **Reference** | : | Hoster, S., 1985. Acute Toxicology – Inhalation, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc. |

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | Limit test |
| **Guideline/method** | : | |
| **Species** | : | Rabbit (albino) |
| **Strain** | : | |
| **Sex** | : | Male and female |
| **Number of animals** | : | 5 of each sex |
| **Vehicle** | : | None |
| **Doses** | : | A single dose of 2.0 g/kg was administered to all animals. |
| **LD50** | : | >2.0 g/kg |
| **Year** | : | 1980 |
| **GLP** | : | Yes (per EPA's proposed GLP regulations at the time) |
| **Test substance** | : | Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4) |
| **Method** | : | Similar to that proposed in 40 CFR 163.81-2 (August 22, 1978). |

| | | |
|---|---|---|
| **Method detail** | : | Animals weighed between 2.0 and 3.0 kg. All animals had their backs clipped free of hair 24 hours prior to testing. All animals had their backs abraded prior to dosing. Test material was applied to the back of each animal and covered with a large gauze patch. An impervious material was then wrapped snugly around the trunk of each animal. The dressings were removed after 24 hours and any excess test material was removed. Animals were observed for a period of 14 days for signs of toxicity. |
| **Result** | : | There were no mortalities in the test. Very substantial skin irritation was noted throughout the observation period, but no other untoward symptoms were observed. Gross pathological examination of all survivors revealed nothing remarkable. |
| **Remark** | : | |
| **Reliability** | : | [2] Reliable with restrictions. Basic data given: comparable to guidelines. |
| **Reference** | : | Biosearch, Inc. (1980). |
| | | |
| **Type** | : | Limit test |
| **Guideline/method** | : | Dermal Toxicity – EPA (40 CFR 163.81-2) |
| **Species** | : | Rabbit (albino) |
| **Strain** | : | New Zealand |
| **Sex** | : | Male and female |
| **Number of animals** | : | 5 of each sex (exposed); 5 of each sex (untreated control) |
| **Vehicle** | : | None |
| **Doses** | : | A single dose of 2.0 g/kg was administered to all animals. |
| **LD50** | : | >2.0 g/kg |
| **Year** | : | 1985 |
| **GLP** | : | No |
| **Test substance** | : | 2% zinc naphthenate, in mineral spirits solvent. Sample density 0.83 g/mL |
| **Method** | : | Dermal Toxicity – EPA (40 CFR 163.81-2) |
| **Method detail** | : | The trunks of the animals were clipped free of hair and abraded prior to dosing. An impervious sleeve was wrapped around the trunk and the dose introduced under the sleeve. At the end of 24 hours, the sleeve was removed, skin reactions noted, and any excess test material removed. Animals were observed for a period of 14 days for signs of toxicity. Weight changes were recorded at 7 days. Gross pathology performed at study termination. |
| **Result** | : | There were no mortalities in the test. All exposed animals exhibited slight erythema at 24 hours and 48 hours. By 72 hours, only 3 animals showed slight erythema and by day 7 all signs of irritation had subsided. No edema was observed. One animal showed weight loss and one showed diarrhea. No other untoward symptoms were observed. Gross pathological examination indicated congested spleen in 2 exposed animals, pale thin spleen in one exposed animal, streak in the liver in one exposed animal, and an abscess under the skin in one animal. All other organs and tissues appeared normal; four autopsied control animals demonstrated normal pathology. |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** No deaths occurred in an acute dermal toxicity study. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively. |
| **Reliability** | : | [2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance. |
| **Reference** | : | Hoster, S., 1985. Acute Toxicology – Dermal, 2% Zinc Naphthenate in |

Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc.

### 5.2.1 SKIN IRRITATION

| | | |
|---|---|---|
| **Type** | : | Primary skin irritation |
| **Guideline/method** | : | |
| **Species** | : | Rabbit (albino) |
| **Strain** | : | |
| **Sex** | : | |
| **Concentration** | : | |
| **Exposure** | : | 0.5 ml of undiluted test material |
| **Exposure time** | : | 24 hr |
| **Number of animals** | : | Six |
| **Vehicle** | : | None |
| **Classification** | : | Study 1: primary skin irritant; Study 2: skin irritant |
| **Year** | : | 1980 |
| **GLP** | : | Yes (per EPA's proposed GLP regulations at the time) |
| **Test substance** | : | Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4) |
| **Method** | : | Similar to that proposed in 40 CFR 163.81-5 (August 22, 1978). |
| **Method detail** | : | The test was conducted twice. After clipping, a 0.5 ml sample of the test material was applied to areas of intact and abraded skin on six albino rabbits for a period of 24 hours. Test material was held in place by gauze patches secured with an impervious material wrapped around the torso of each animal. Examination and scoring (Draize method) for erythema, eschar, and edema was conducted at 24 and 72 hours. |
| **Result** | : | Results were similar for both intact and abraded skin and at both time points. Scores were similar for the primary endpoints. The primary irritation scores were 6.29 and 4.29 for the first and second tests, respectively. |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** Moderately to severely irritating to rabbits. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively |
| **Reliability** | : | [2] Reliable with restrictions. Basic data given: comparable to guidelines. |
| **Reference** | : | Biosearch, Inc. (1980). |

| | | |
|---|---|---|
| **Type** | : | Primary skin irritation |
| **Guideline/method** | : | Skin Irritation Test – EPA (40 CFR 163.81-5) |
| **Species** | : | Rabbit (albino) |
| **Strain** | : | New Zealand |
| **Sex** | : | Not specified |
| **Concentration** | : | |
| **Exposure** | : | 0.5 ml of undiluted test material |
| **Exposure time** | : | 24 hr |
| **Number of animals** | : | Six |
| **Vehicle** | : | |
| **Classification** | : | Slight irritation at 72 hours but subsided by 96 hours |
| **Year** | : | 1985 |
| **GLP** | : | No |
| **Test substance** | : | 2% zinc naphthenate, solvent. |
| **Method** | : | Skin Irritation Test – EPA (40 CFR 163.81-5) |
| **Method detail** | : | The trunk of each animal was clipped free of hair. After clipping, a 0.5 ml |

| | | |
|---|---|---|
| | | sample of the test material was applied to two areas of intact and two areas of abraded skin on six albino rabbits for a period of 24 hours. Test material was held in place by gauze patches secured with an impervious material wrapped around the torso of each animal. Examination and scoring for erythema, eschar, and edema was conducted at 24, 72 and 96 hours. |
| **Result** | : | At 24 hours, no erythema was observed but two animals had slight to moderate edema on abraded skin. At 72 hours, 5 animals exhibited slight erythema but no animals exhibited edema. By 96 hours, all signs of irritation had subsided. |
| **Remark** | : | |
| **Reliability** | : | [2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance. |
| **Reference** | : | Hoster, S., 1985. Acute Toxicology – Skin Irritation, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc. |

### 5.2.2 EYE IRRITATION

| | | |
|---|---|---|
| **Type** | : | Primary eye irritation |
| **Guideline/method** | : | |
| **Species** | : | Rabbit (albino) |
| **Strain** | : | New Zealand White |
| **Sex** | : | Not specified |
| **Concentration** | : | |
| **Dose** | : | 0.1 ml of undiluted test material |
| **Exposure time** | : | |
| **Number of animals** | : | Six |
| **Vehicle** | : | None |
| **Classification** | : | Not a primary ocular irritant |
| **Year** | : | 1980 |
| **GLP** | : | Yes (per EPA's proposed GLP regulations at the time) |
| **Test substance** | : | Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4) |
| **Method** | : | Similar to that proposed in 40 CFR 163.81-4 (August 22, 1978). |
| **Method detail** | : | A 0.1 ml sample of the material was instilled into the right eyes of six adult rabbits. Left eyes were untreated and served as controls. The test material was not washed from the eyes. The treated eyes were examined and scored according to Draize scale at one, two, three, five, and seven days following instillation of the test material. |
| **Result** | : | Total ocular irritation scores ranged from 4 to 8 (avg. = 7.0) for individual animals at 24 hours after instillation. Total ocular irritation scores were zero for all animals at all subsequent time points. |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** Raw naphthenic acid derived from kerosene was judged to be an irritant. In a later summary report, eye irritation was judged to be moderate |
| **Reliability** | : | [2] Reliable with restrictions. Basic data given: comparable to guidelines. |
| **Reference** | : | Biosearch, Inc. (1980). |

| | | |
|---|---|---|
| **Type** | : | Primary eye irritation |
| **Guideline/method** | : | Skin Irritation Test – EPA (40 CFR 163.81-4 proposed) |
| **Species** | : | Rabbit (albino) |
| **Strain** | : | |
| **Sex** | : | Not specified |
| **Concentration** | : | |
| **Dose** | : | 0.1 ml of undiluted test material |
| **Exposure time** | : | |
| **Number of animals** | : | Nine (6 exposed and 3 control) |

| | | |
|---|---|---|
| **Vehicle** | : | None |
| **Classification** | : | Not an irritant |
| **Year** | : | 1985 |
| **GLP** | : | No |
| **Test substance** | : | 2% zinc naphthenate, solvent. |
| **Method** | : | Skin Irritation Test – EPA (40 CFR 163.81-4 proposed) |
| **Method detail** | : | A 0.1 ml sample of the material was instilled into the right eyes of six adult rabbits. In these six animals, the test material was not washed from the eyes. Left eyes were untreated and served as controls. In three other adult rabbits, the test material was instilled in the same manner but each eye was subsequently flushed with lukewarm water no sooner than 20-30 seconds after instillation. The treated eyes were examined and scored for damage to the cornea, iris and conjunctiva at 1, 2, 3, 4 and 7 days after treatment. |
| **Result** | : | All ocular irritation scores were zero at all time points. No irritation was observed. |
| **Remark** | : | |
| **Reliability** | : | [2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance. |
| **Reference** | : | Hoster, S., 1985. Acute Toxicology – Eye Irritation, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc. |

### 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | 90-day dermal toxicity |
| **Guideline/method** | : | FIFRA 82-3 and OECD 411 |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | Male and female |
| **Number of animals** | : | 10 of each sex per treatment group |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | 6 hours per day for 13 weeks |
| **Frequency of treatment** | : | Once per day; 5 days per week for 13 weeks |
| **Post exposure period** | : | None |
| **Doses** | : | 100, 300, and 1000 mg/kg/day |
| **Control group** | : | Yes |
| **NOAEL** | : | 300 mg/kg/day excluding dermal irritation as an endpoint |
| **LOAEL** | : | 1,000 mg/kg/day excluding dermal irritation as an endpoint |
| **Other** | : | Dermal irritation was present at the application site in all groups, including control. Irritation increased in a dose-related manner. |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (Purity = 98.9%; 14.3% zinc) |
| **Method** | : | |
| **Method detail** | : | Test substance was dissolved in light mineral oil at a concentration of 50% by weight and administered onto the clipped intact dorsal skin (right flank) of each animal. After application, each test site was wrapped with a gauze binder and the dressing secured with Deriform® tape. At the end of a 6-hour exposure period, the dressings were removed and the test sites were wiped with disposable paper towels moistened with mineral oil. The concurrent control group received the vehicle (mineral oil) on a comparable regimen at a dose volume equal to the amount of vehicle received by the highest dose group. |
| **Result** | : | No treatment-related clinical signs or effects on mortality were apparent in the study; however, dermal irritation (including moderate and severe grades of erythema and edema, as well as fissuring) was observed in a dose- |

related manner. Severe signs of skin irritation such as eschar and blanching were not observed. A tolerance developed to the irritating effects of the test substance and the incidences of severe edema, erythema and fissuring were lower during the final weeks of the study. Histopathologic evaluation of the application sites revealed treatment-related lesions characterized by hyperkeratosis of the epidermal surface and dermal hyperplasia. Body weight means of both male and female rabbits in the 1000 mg/kg/day group were lower than control means throughout the study. Relative mean kidney and adrenal weights of the high dose group's animals were significantly above the control mean. No treatment-related effects were apparent in the serum chemistry values. A slight increase in neuturophils in the high dose group was the only alteration in clinical pathological parameters indicative of a treatment-related effect.

**Remark** :
**Reliability** : (1) Reliable without restrictions.
**Reference** : Tomkins, E.C. 1990. 90-Day dermal study in rabbits with zinc naphthenate. WIL Research Laboratories. Lab Study No. WIL-153006.

**Type** : Contact dermal irritation / Sensitization
**Guideline/method** :
**Species** : Guinea pig (albino)
**Strain** :
**Sex** : Male
**Number of animals** : 10
**Route of admin.** : Dermal
**Exposure period** : See method details below
**Frequency of treatment** : See method details below
**Post exposure period** : See method details below
**Dose** : 0.5 ml of 10% w/v suspension in mineral spirits
**Control group** : None
**NOAEL** :
**LOAEL** :
**Other** :
**Year** : 1980
**GLP** : Yes (per EPA's proposed GLP regulations at the time)
**Test substance** : Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
**Method** :
**Method detail** : A 0.5 ml sample of test material was applied to intact skin test sites on 10 guinea pigs. A gauze patch was used to hold the test substance in place. After a 24-hour contact period, the patch was removed and the animals were allowed to rest for one day. Following the rest period, another application was applied to the same skin site using a fresh sample. This sequence was repeated for a total of ten induction applications. After the tenth application, the animals were rested for a two-week period. Following this period, a challenge application was placed at skin sites differing from the original test sites. The challenge application was removed after 24 hours. Sites were examined for irritation using the Draize scale 24 hours after each induction application, and 24 and 48 hours after the challenge application.
**Result** : The test material produced well defined erythema and very slight edema during the induction period. Similar or slightly less severe effects were noted after the challenge dose. Based on study results, the test material appeared to be a primary skin irritant and fatiguing agent, and possibly a sensitizing agent in the guinea pig.
**Remark** :
**Reliability** : [2] Reliable with restrictions. Basic data given: comparable to guidelines.

| | | |
|---|---|---|
| **Reference** | : | Biosearch, Inc. (1980). |

## 5.5 GENETIC TOXICITY 'MUTAGENICITY'

| | | |
|---|---|---|
| **Type** | : | L5178Y (TK+/TK-) Mouse lymphoma mutagenesis |
| **Guideline/method** | : | FIFRA 84-2 |
| **System of testing** | : | Suspension / plate |
| **Species** | : | Mouse |
| **Strain** | : | L5178Y (TK+/TK-) |
| **Test concentrations** | : | Initial assay: 1.3 to 100 µg/ml;<br>Confirmatory assay: 7.5 to 75 µg/ml |
| **Cytotoxic concentr.** | : | 100 µg/ml for nonactivated cultures; 1000 µg/ml for activated cultures |
| **Metabolic activation** | : | Rat liver S-9 fraction, induced with Aroclor 1254 |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (Purity = 98.9%; 14.3% Zn) |
| **Method** | : | Clive and Spector, 1975 (Mutation Res. 31:17-29) |
| **Method detail** | : | Ethanol was used as the solvent for preparing dilutions of the test substance. |
| **Result** | : | Positive findings (mutant frequencies at least twice the frequency of the controls), both with and without metabolic activation, were found in the initial and confirmatory assays. A dose-dependent response was seen in the treated cultures both with and without metabolic activation. Colony sizing data indicated an increase in the proportion of small mutant colonies from cultures treated with the test substance, suggesting that it may show clastogenic activity. All criteria for a valid test were met. |
| **Remark** | : | **Supporting data for similar salts:** Similar mouse lymphoma tests with the calcium and copper salts of naphthenic acids were also positive both with and without metabolic activation. However, copper naphthenate produced negative results in the Ames Assay with *Salmonella typhimurium* both with and without metabolic activation. (Reference: Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute, Dr. David Longfellow, Project Officer. Cited in Chemical Carcinogenesis Research Information System, National Library of Medicine: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS) |
| **Reliability** | : | (1) Reliable without restrictions. |
| **Reference** | : | Harbell, H.W. 1990. L5178Y TK+/- mouse lymphoma mutagenesis assay with confirmation – test article zinc naphthenate. Microbiological Associates, Inc. Lab Study No. T9036.701. |

| | | |
|---|---|---|
| **Type** | : | Unscheduled DNA Synthesis |
| **Guideline/method** | : | FIFRA 84-4 |
| **System of testing** | : | Primary hepatocytes |
| **Species** | : | Rat |
| **Strain** | : | Harlan Sprague-Dawley |
| **Test concentrations** | : | 0.015 to 35 µg/ml (8 dose levels) |
| **Cytotoxic concentr.** | : | 15 µg/ml |
| **Metabolic activation** | : | No |
| **Year** | : | 1989 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (Purity = 98.9%; 14.3% Zn) |
| **Method** | : | Williams, 1979 (In Chemical Mutagens, Vol. VI, DeSerres, F.J. and A. Hollander, eds., Plenum Press, pp 61-79) |
| **Method detail** | : | Ethanol was used to dissolve the test substance and as a solvent control. DMBA was used as a positive control. A parallel cytotoxicity test was conducted to determine the relative toxicity of the test substance. |

| | | |
|---|---|---|
| **Result** | : | The test substance did not cause a significant increase in unscheduled DNA synthesis as measured by the mean number of net nuclear grain counts at any dose level. All criteria for a valid test were met. |
| **Remark** | : | |
| **Reliability** | : | (1) Reliable without restriction. |
| **Reference** | : | Curren, R.D. 1989. Unscheduled DNA synthesis in rat primary hepatocytes - test article zinc naphthenate. Microbiological Associates, Inc. Lab Study No. T9036.380. |

### 5.6 GENETIC TOXICITY 'CHROMESOMAL ABERRATION

| | | |
|---|---|---|
| **Type** | : | Chromosome aberration assay |
| **Guideline/method** | : | FIFRA 84-2 |
| **System of testing** | : | Chinese hamster ovary cells |
| **Species** | : | Hamster |
| **Strain** | : | Chinese |
| **Test concentrations** | : | Initial assay: 5 to 80 μg/ml for nonactivated cultures;10 to 160 μg/ml for activated cultures;<br>Confirmatory assay: 80 to 200 μg/ml for nonactivated cultures; 60 to 140 μg/ml for activated cultures |
| **Cytotoxic concentr.** | : | 80 μg/ml |
| **Metabolic activation** | : | Yes, with Aroclor induced S-9 fraction from male Sprague-Dawley rats |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (Purity = 98.9%; 14.3% Zn) |
| **Method** | : | |
| **Method detail** | : | Ethanol was used to dissolve the test substance and as a solvent control. Triethylenemelamine and cyclophosphamide were used as positive controls. Whenever possible, a minimum of 100 metaphase spreads (50 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. |
| **Result** | : | Zinc naphthenate produced positive results in the CHO cytogenetics assay. Toxicity was a limiting factor in the analysis of test concentrations in both the nonactivated and S-9 activated studies. The percentage of cells with structural chromosome aberrations was significantly increased, in a dose-responsive manner, at all test concentrations analyzed for both the S-9 activated and the nonactivated test systems. |
| **Remark** | : | |
| **Reliability** | : | (1) Reliable without restriction. |
| **Reference** | : | Putman, D.L. and M.J. Morris. 1990. Chromosome aberrations in Chinese hamster ovary (CHO) cells - test article zinc naphthenate. Microbiological Associates, Inc. Lab Study No. T9036.337. |

### 5.8.2 DEVELOPMENTAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | Teratology / developmental toxicity |
| **Guideline/method** | : | |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Female |
| **Route of admin.** | : | Oral |
| **Exposure period** | : | Day 6 through 15 of gestation |
| **Frequency of treatment** | : | Daily |
| **Duration of test** | : | Mating until day 20 of gestation |
| **Doses** | : | 94, 188, and 938 mg/kg/day |
| **Control group** | : | Yes (received 3.75 mL/kg/day of corn oil) |

| | | |
|---|---|---|
| **NOAEL maternal tox.** | : | 188 mg/kg/day |
| **NOAEL teratogen.** | : | 188 mg/kg/day |
| **Other** | : | LOAEL was 938 mg/kg/day for maternal toxicity |
| **Other** | : | LOAEL was 938 mg/kg/day for toxicity to fetuses |
| **Other** | : | |
| **Year** | : | 1991 |
| **GLP** | : | Yes |
| **Test substance** | : | Zinc naphthenate, technical, containing 13.7% zinc. Dosed in corn oil. |
| **Method** | : | Standing Operating Procedure No. 25, Teratology Study in Rats, July 1981, Toxicology Division, U.S. Army Environmental Hygiene Agency. |
| **Method detail** | : | Doses were set based on results of a pilot study. There were at least 33 positively mated females in each dose group. Females were sacrificed on day 20 of gestation. Each uterus was exposed and counts were made of corpora lutea, implantation sites, resorptions, and fetuses. Fetuses were preserved and examined for either skeletal (even-numbered fetuses) or soft tissue (odd numbered fetuses) malformations. |
| **Result** | : | Oral administration of zinc naphthenate to rats during the major period of organogenesis did not result in teratogenic effects. Transient maternal toxicity was confined to the highest dosage group (938 mg/kg/day) and consisted of lethargy and lower body weight gain. Maternal treatment at that dosage level also produced a higher incident of resorptions and lower average fetal body weights. Dams receiving zinc naphthenate at either 94 or 188 mg/kg/day were not adversely affected, nor were their developing fetuses. Compared to controls, there was an increase in the incidence of variants (minor morphological deviations) in all treatment groups; however, there was not a dose-response for this effect. It was concluded that zinc naphthenate is not teratogenic and does not cause developmental toxicity at doses that are not maternally toxic. |
| **Remark** | : | |
| **Reliability** | : | [1] Reliable without restriction. Comparable to guideline study. |
| **Reference** | : | Angerhofer, R.A., M.W. Michie, M.P. Barlow, and P.A. Beall. 1991 Phase 4, Toxicological Study No. 75-51-0497-91, Assessment of the developmental toxicity of zinc naphthenate in rats, June 1985 – July 1988. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD. NTIS No. ADA235308. |
| **Type** | : | Oral administration |
| **Guideline/method** | : | FIFRA 83-3 |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley Crl:CDBR |
| **Sex** | : | Female |
| **Route of admin.** | : | Oral |
| **Exposure period** | : | Day 6 through 15 of gestation |
| **Frequency of treatment** | : | Daily |
| **Duration of test** | : | Mating until day 20 of gestation |
| **Doses** | : | 50, 250, and 500 mg/kg/day |
| **Control group** | : | Yes (received 10 mL/kg/day of corn oil) |
| **NOAEL maternal tox.** | : | 250 mg/kg/day (excluding marginal clinical signs) |
| **NOAEL teratogen.** | : | 500 mg/kg/day |
| **Other** | : | LOAEL was 500 mg/kg/day for maternal toxicity (based on clinical signs and slightly reduced food consumption) |
| **Other** | : | LOAEL for fetuses was above the highest dose tested |
| **Other** | : | |
| **Year** | : | 1990 |
| **GLP** | : | Yes |
| **Test substance** | : | Technical grade zinc naphthenate (purity = 98.9%; 14.3% Zn). |

| | | |
|---|---|---|
| **Method** | : | Oral gavage |
| **Method detail** | : | Doses were set based on results of a range-finding study. The test substance was dissolved in corn oil and administered by gastric gavage at a dose volume of 10 ml/kg. There were 25 positively mated females in each dose group. Females were sacrificed on day 20 of gestation for a scheduled Cesarean section. The uteri and ovaries were examined and the location and numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Fetuses were weighed, sexed, and examined for external, skeletal and soft tissue malformations and developmental variations. |
| **Result** | : | Maternal survival was not adversely affects in the study and no indication of maternal toxicity was apparent at a dose level of 50 mg/kg/day. Clinical signs of toxicity observed in the high dose females included anogenital and/or urogenital staining, staining around the mouth, and salivation. Some of the same clinical signs were also seen in females at the mid-dose level, although the incidence was greatly reduced. No adverse effects were apparent on body weight data or gravid uterine weight data although food consumption was slightly reduced in the high dose group. Intrauterine growth and survival were not adversely affected at any of the treatment levels. The nature and frequency of fetal malformations and developmental variations expressed appeared to be spontaneous in origin. |
| **Remark** | : | **Supporting data for dissociation products:**<br>**Acid:** Results are highly consistent with the developmental toxicity study conducted on zinc naphthenate by the U.S. Army Environmental Hygiene Agency (see above). |
| **Reliability** | : | [1] Reliable without restriction. Comparable to guideline study. |
| **Reference** | : | Nemec, M.D. 1990. A developmental toxicity study of zinc naphthenate in rats. WIL Research Laboratories, Inc. Lab Study No. WIL-153004. |

### 5.8.3 TOXICITY TO REPRODUCTION

| | | |
|---|---|---|
| **Type** | : | Two generation, oral administration |
| **Guideline/method** | : | |
| **In vitro/in vivo** | : | In vivo |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male and female |
| **Route of admin.** | : | Diet |
| **Exposure period** | : | Two generations |
| **Frequency of treatment** | : | Continuous in diet |
| **Duration of test** | : | Through weaning of second (F2) generation of offspring |
| **Doses** | : | 500, 1000, or 5000 ppm in diet |
| **Control group** | : | Yes |
| **Year** | : | 1991 |
| **GLP** | : | Yes |
| **Test substance** | : | Zinc naphthenate, technical, containing 13.7% zinc. Dosed in corn oil. |
| **Method** | : | Standing Operating Procedure, Reproduction Study in Rats, August 1986 revision, Toxicology Division, U.S. Army Environmental Hygiene Agency. |
| **Method detail** | : | Rats were fed zinc naphthenate for 10 weeks prior to mating of the parental (P) generation. Feeding of the treated diet was continued during mating, gestation, and lactation for both the P and F1 generations. Body weights and feed consumption were measured three times per week during the exposure period. Animals were checked daily for toxic signs. After sacrifice, animals were examined grossly and target organ tissues removed for histopathologic examination. Individual body weights, abnormalities, |

mortalities, and total litter weights for F1 pups were noted on days 0, 4, 7, 14, and 21 post partum.

**Results** : The continuous diets of zinc naphthenate employed in the study produced no adverse effects on reproductive function of rats over two generations. Rats fed a diet of 5,000 ppm experienced a significant weight loss (or reduced weight gain), but this effect had no subsequent effect on mating or viability of offspring over two generations. It is concluded that zinc naphthenate does not produce adverse effects on reproduction at dietary levels that are not maternally or paternally toxic. The NOAEL for all endpoints in this study was 1,000 ppm in the diet.

**Remark** :

**Reliability** : [1] Reliable without restriction. Comparable to guideline study.

**Reference** : Michie, M.W., Angerhofer, R.A., M.P. Barlow, and P.A. Beall. 1991 Phase 5, Effects of ingestion of zinc naphthenate on reproductive function of rats, Toxicological Study No. 75-51-0497-91. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD. NTIS No. ADA235224.

## 6.0 OTHER INFORMATION

### 6.1 Carcinogenicity

No adequate experimental evidence has been found to indicate that zinc salts administered orally or parenterally are tumorigenic. (WHO, 2001, Environmental Health Criteria 221, Zinc).

### 6.2 Skin sensitization

Zinc sulfate is not a skin sensitizer in animals. (Risk Assessment for Zinc Metal, 2001, draft).

# 1. General Information

**ID** 1338-24-5

**Date** December 15, 2005

## 1.0 SUBSTANCE INFORMATION



| | | |
|---|---|---|
| **Generic Name** | : | |
| **Chemical Name** | : | Naphthenic acids |
| **CAS Registry No.** | : | 1338-24-5 |
| **Component CAS Nos.** | : | |
| **EINECS No.** | : | 215-662-8 |
| **Structural Formula** | : | |
| **Additional description** | : | Naphthenic acid is mixture of various carboxylic acids which occur naturally in crude petroleum. The most common class of acid is derived from cyclopentane and has the general formula CnH2n-202, where n = 8 to 12. This basic cyclopentane structure can be more or less highly alkylated. Other classes of acids include simple paraffinic acids of the general formula CnH2n02 where n = 5 to 8, and acids with larger more complicated molecules of the general formula CnH2-402, where n = 13 to 23. The classes and proportions of individual naphthenic acids in the overall mix vary according to the origin of the crude oil. |
| **Molecular Weight** | : | Generally between 140 and 450 |
| **Synonyms and Tradenames** | : | |
| **References** | : | AGS Chemicals Ltd., 2003, Product Information, Naphthenic Acid; Headley, J.V. and D.W. McMartin, 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, A39(8):1989 -2010. |

## 2.1 MELTING POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | -35 to +2°C |
| **Decomposition** | : | at °C |
| **Sublimation** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Commercially available naphthenic acid |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | A range of melting points would be expected, based upon the hydrocarbon composition of the specific naphthenic acid mixture. Estimated melting points were calculated for one to four ring cycloalkyl naphthenic acid structures with molecular weights ranging from 260 to 320; these dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands. Melting points calculated using EPIWIN v3.10 ranged from 117ºC to 160ºC for these structures (Appendix C). In contrast, structural profiles of commercial naphthenic acids have been shown to differ substantially from natural extracts (Rogers et al., 2002, cited in Appendix C). Product literature for commercially available naphthenic acid provides a melting point range of -35º to +2ºC (AGS Chemicals Ltd., 2005). |
| **Reliability** | : | |
| **Reference** | : | API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C); AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid (). |

## 2.2 BOILING POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | 140ºC to 200ºC |
| **Decomposition** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Commercially available naphthenic acid |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | A range of boiling points would be expected, based upon the hydrocarbon composition of the specific naphthenic acid mixture. Estimated boiling points were calculated for one to four ring cycloalkyl naphthenic acid structures with molecular weights ranging from 260 to 320; these dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands. Boiling points calculated using EPIWIN v3.10 ranged from 233ºC to 375ºC for these structures (Appendix C). In contrast, structural profiles of commercial naphthenic acids have been shown to differ substantially from natural extracts (Rogers et al., 2002, cited in Appendix C). Product literature for commercially available naphthenic acid provides a boiling point range of 140º to 200ºC (AGS Chemicals Ltd., 2005). |
| **Reliability** | : | |
| **Reference** | : | API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C); AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid. |

## 2.3 DENSITY

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | 0.91 to 0.96 g/cm$^3$ at 15ºC |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | | AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid (http://www.ags-chemicals.com) |

## 2.4 VAPOR PRESSURE

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | |
| **Decomposition** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | It was estimated using EPIWIN v.310 that the vapor pressures of the components of naphthenic acid mixtures would be near or below the measurable limits cited in standard guideline methods and thus, the total vapor pressure of naphthenic acids is expected to be exceedingly low (Appendix C) |
| **Reliability** | : | |
| **Reference** | : | API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C) |

## 2.5 PARTITION COEFFICIENT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Partition coefficient** | : | |
| **Log Pow** | : | |
| **pH value** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Using EPIWIN v3.10, partition coefficients were estimated for a range of molecular weight naphthenic acids spanning the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts. Resulting log Kow values ranged from 5.1 to 9.2. Mixtures of naphthenic acids with a significant proportion of structures with molecular weights below 250 will likely show lower log Kow values than those |

presented. (Appendix C)

**Reliability** :

**Reference** : API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C)

### 2.6.1 SOLUBILITY IN WATER

**Type** :
**Guideline/method** :
**Value** : at °C
**pH value** :
**concentration** : at °C
**Temperature effects** :
**Examine different pol.** :
**PKa** : at °C
**Description** :
**Stable** :
**Deg. product** :
**Year** :
**GLP** :
**Test substance** :
**Deg. products CAS#** :
**Method** :
**Method detail** :
**Result** :
**Remark** : Using EPIWIN v3.10, water solubility was estimated for a range of molecular weight naphthenic acids spanning the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts. Resulting water solubility estimates ranged from 0.0003 to 2.1 mg/L. Mixtures of naphthenic acids with a significant proportion of structures with molecular weights below 250 will likely show greater water solubilities than those presented. (Appendix C)

**Reliability** :

**Reference** : API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C);

### 2.7 FLASH POINT

**Type** :
**Guideline/method** :
**Value** :
**Year** :
**GLP** :
**Test substance** :
**Method** :
**Method detail** :
**Result** :
**Remark** :
**Reliability** :
**Reference** : .

### 3.1.1 PHOTODEGRADATION

| | | |
|---|---|---|
| **Type** | | |
| **Guideline/method** | : | |
| **Light source** | : | |
| **Light spectrum** | : | |
| **Relative intensity** | : | based on |
| **Spectrum of substance** | : | lambda (max, >295nm) : |
| | | epsilon (max) : |
| | | epsilon (295) : |
| **Conc. of substance** | : | at °C |
| **DIRECT PHOTOLYSIS** | | |
| **Halflife (t1/2)** | : | |
| **Degradation** | : | % after |
| **Quantum yield** | : | |
| **INDIRECT PHOTOLYSIS** | | |
| **Sensitizer** | : | |
| **Conc. of sensitizer** | : | |
| **Rate constant** | : | |
| **Degradation** | : | |
| **Deg. product** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Three naphthenic acid mixtures (two commercially-available and one extracted from an Athabasca Oil Sands tailings pond) as well as three individual naphthenic acids: 4-methylcyclohexaneacetic acid (4-MCHAA), 4-methylcyclohexanecarboxylic acid (4-MCHCA), and 3-methylcyclohexane-carboxylic acid (3-MCHCA). |
| **Deg. products CAS#** | : | |
| **Method** | : | |
| **Method detail** | : | Experiments were conducted with natural sunlight, artificial solar radiation in growth chambers using an incandescent and fluorescent lamp canopy, artificial UV-range solar radiation in quartz annular photochemical cells, and UV-254 ultraviolet lamps in quartz annular photochemical cells. All aqueous solutions of naphthenic acids were prepared in Athabasca Rivver water and 1 mL aliquots collected at selected time intervals to assess photochemical degradation as well as toxicity changes. Concentrations were 0.5 to 125 mg/L depending upon the compound or mixture under study. Control reactors were monitored simultaneously in the absence of UV light in natural water and in both the absence and presence of UV light in reagent water. The production of hydroxyl radicals during photolysis was measured with a benzoic acid (BA) chemical probe. As BA is lost and 3-hydrobenzoic acid (HBA) formed when the hydroxyl radical is scavenged, the hydroxyl radical concentration is calculated and the primary method of photolysis determined (e.g., indirect or direct). Benzoic acid was added to selected samples at a concentration of 6.4 mg/L. Loss of BA and production of HBA was measured using LC/MS. The concentration of the naphthenic acids was also measured using LC/MS. |
| **Result** | : | Naphthenic acid photolysis resulting from exposure to natural and artificial sunlight was limited. After one week of exposure to natural solar radiation, no individual compounds or mixtures were significantly degraded, although compositional changes were noted in the mixtures. Artificial solar radiation was similarly ineffective. Exposure to UV-245 radiation induced the most photolysis, but was only particularly effective on 4-MCHAA (half-life 3.2 – 3.6 hours) and was not an efficient means for complete removal of the other individual acids or complex mixtures from natural waters. |

| | | |
|---|---|---|
| **Remark** | : | |
| **Reliability** | : | (1) Reliable with restrictions. Not a guideline study, but sufficiently documented to provide useful information. |
| **Reference** | : | McMartin, D.W., J.V. Headley, D.A. Friesen, K.M. Peru, and J.A. Gillies, 2004. Photolysis of naphthenic acids in natural surface water, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, A39(6):1361-1383. |

### 3.1.2 DISSOCIATION

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **pKa** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Approx. water solubility** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Naphthenic acids exist as weak acids, with most pKa values being reported at about 5. At low pHs, they exist in their undissociated form and tend to partition onto solids. At high pHs, they exist in their dissociated form and become more mobile. (Appendix C) |
| **Reliability** | : | |
| **Reference** | : | |

### 3.2.1 MONITORING DATA

| | | |
|---|---|---|
| **Type of measurement** | : | |
| **Media** | : | |
| **Concentration** | : | mg/l |
| **Substance measured** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

### 3.3.1 TRANSPORT (FUGACITY)

| | | |
|---|---|---|
| **Type** | : | |
| **Media** | : | |
| **Air** | : | % (Fugacity Model Level I) |
| **Water** | : | % (Fugacity Model Level I) |
| **Soil** | : | % (Fugacity Model Level I) |
| **Biota** | : | % (Fugacity Model Level II/III) |
| **Soil** | : | % (Fugacity Model Level II/III) |
| **Year** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Using EPIWIN v3.10, Level I fugacity modeling was performed for a range of naphthenic acids covering the predominant molecular weight and ring |

structures reported to predominate in Athabasca oil sands extracts. The principal distribution of these constituents following environmental release would be to soil and/or sediment, with overwhelming (98%) partitioning to soil. (Appendix C)

**Reliability** :
**Reference** :

### 3.5 BIODEGRADATION

**Type** :
**Guideline/method** : Non-guideline study
**Inoculum** : Sodium naphthenate-degrading enrichment cultures derived from oil sands tailings water.
**Concentration** : related to
related to
**Contact time** :
**Degradation** : (±) % after day(s)
**Result** :
**Kinetic of test subst.** : 50% converted to $CO_2$ in a 24-d period.
%
%
%
%
**Control substance** :
**Kinetic** : %
%
**Deg. product** :
**Year** : 1994
**GLP** :
**Test substance** : Commercial sodium naphthenate mixture
**Deg. products CAS#** :
**Method** :
**Method detail** :
**Result** : Commercial mixtures of the sodium salts of naphthenic acids were shown to degrade and mineralize to $CO_2$ when inoculated with microbial populations indigenous to oil sands tailings. Approximately 50% of the organic carbon was converted to $CO_2$ over a 24-d period. Three of four model naphthenic acid compounds were also degraded by the enrichment cultures, with approximately 40-50% of the organic carbon converted to $CO_2$ over a 24-d period.
**Remark** : Additional studies by Clemente et al. (2004) monitored the concentration and composition of naphthenic acids in aerobic biodegradation studies using sodium salts of naphthenic acids. Within 10 days of incubation with enrichment cultures on naphthenic acid-degraders, naphthenic acids concentration dropped from about 100 to <10 mg/L, accompanied by release of about 60% of the carbon as $CO_2$. GC/MS results indicated that the lower molecular weight acids (n = 5-13) were degraded more readily than high molecular weight acids. Clemente, J.S., M.D. Mackinnon, and P.M. Fedorak, 2004. Aerobic biodegradation of two commercial naphthenic acids preparations, Environ. Sci. Technol. 38:1009 – 1016.
**Reliability** :
**Reference** : Herman et al. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can. J. Microbiol. 40:467-477; Appendix C

## 3.7 BIOCONCENTRATION

| | | | |
|---|---|---|---|
| **Type** | : | | |
| **Guideline/method** | : | | |
| **Species** | : | | |
| **Exposure period** | : | at | °C |
| **Concentration** | : | | |
| **BCF** | : | | |
| **Elimination** | : | | |
| **Year** | : | | |
| **GLP** | : | | |
| **Test substance** | : | | |
| **Method** | : | | |
| **Method detail** | : | | |
| **Result** | : | | |
| **Remark** | : | | |
| **Reliability** | : | | |
| **Reference** | : | | |

## 4.1 ACUTE TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Exposure period** | : | |
| **NOEC** | : | |
| **LC0** | : | |
| **LC50** | : | 5.6 – 7.1 mg/L for bluegill |
| **LC100** | : | |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Data in the U.S. EPA ECOTOX database from three references indicate an 96-h LC50 range for naphthenic acids of 5.6 – 7.1 mg/L for bluegill. The 96-h LC50 for another fish species, the zebra fish (*Danio rerio*), is reported as 16.3 mg/L for naphthenic acids. (U.S. Environmental Protection Agency. 2005. ECOTOX Database System. http://www.epa.gov/ecotox). Further information about these studies, and several additional references, is given in Appendix C. Commercial sodium salts of naphthenic acid produced LC50 values of 50 mg/L for kutum (*Rutulis frisii* kutum) and sturgeon (*Acipenser gueldenstaedi*) and 75 mg/L for roach (*Rutulis rutulis* caspicus) (Dokholyan and Magomedov, 1983, cited in Rogers, V.V., et al., Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355). |
| **Reliability** | : | |
| **Reference** | : | |

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Exposure period** | : | |
| **NOEC** | : | |
| **EC0** | : | |
| **EC50** | : | |
| **EC100** | : | |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |

| | | |
|---|---|---|
| **Result** | : | |
| **Remark** | : | A 96-h LC50 of 4.8 mg/L for calcium naphthenate has been reported for the marine copepod, *Nitocra spinipes*. (Bengtsson, B.E. and M. Tarkpea. 1983. The acute aquatic toxicity of some substances carried by ships. Mar. Pollut. Bull. 14:213-214). The zooplankton species Nephargoides maeoticus tolerated naphthenic acids concentrations up to only 0.15 mg/L (Dokholyan and Magomedov, 1984, cited in Clemente, J.S. and P.M. Fedorak, 2005, A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere 60:585-600). |
| **Reliability** | : | |
| **Reference** | : | |

### 4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Endpoint** | : | |
| **Exposure period** | : | |
| **NOEC** | : | |
| **LOEC** | : | |
| **EC0** | : | |
| **EC10** | : | |
| **EC50** | : | |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | The toxicity of naphthenic acids to populations of the freshwater diatom, *Navicula seminulum*, has been measured. The 96-h EC50 for growth ranged from 26.0 – 80.5 mg/L (Academy of Natural Sciences. 1960. Cited in the EPA ECOTOX Database 2005. http://www.epa.gov/ecotox). |
| **Reliability** | : | |
| **Reference** | : | |

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

| | |
|---|---|
| **In vtro/in vivo** | : |
| **Type** | : |
| **Guideline/method** | : |
| **Species** | : |
| **Number of animals** | : |
| **Males** | : |
| **Females** | : |
| **Doses** | : |
| **Males** | : |
| **Females** | : |
| **Vehicle** | : |
| **Route of administration** | : |
| **Exposure time** | : |
| **Product type guidance** | : |
| **Decision on results on acute tox. tests** | : |
| **Adverse effects on prolonged exposure** | : |
| **Half-lives** | : 1st.<br>2nd.<br>3rd. |
| **Toxic behavior** | : |
| **Deg. product** | : |
| **Deg. products CAS#** | : |
| **Year** | : |
| **GLP** | : |
| **Test substance** | : |
| **Method** | : |
| **Method detail** | : |
| **Result** | : |
| **Remark** | : |
| **Reliability** | : |
| **Reference** | : |

### 5.1.1 ACUTE ORAL TOXICITY

| | |
|---|---|
| **Type** | : Acute oral LD50 |
| **Guideline/Method** | : |
| **Species** | : Rat |
| **Strain** | : Wistar |
| **Sex** | : Male |
| **Number of animals** | : 5 per dose level (7 dose levels) |
| **Vehicle** | : None - administered undiluted |
| **Doses** | : 1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg bw |
| **LD50** | : 5.88 g/kg bw (4.31 - 8.02 g/kg bw) |
| **Year** | : 1979 |
| **GLP** | : unknown |
| **Test substance** | : MRD-79-10 (raw naphthenic acid derived from kerosene) |
| **Method** | : |
| **Method detail** | : Rats were observed at 1, 2, 4 and 6 hours after dosing and then daily for 14 days. Mortality, toxicity, and pharmacological effects were recorded. Body weights were recorded at pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross |

pathology.

| | | |
|---|---|---|
| **Result** | : | Deaths occurred at dose levels of 3.16 g/kg and higher. Significant pre-death toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died included dilated hearts and gastrointestinal irregularities. |
| **Remark** | : | Other data for rats includes an LD50 of 3.0 g/kg bw for naphthenic acid fraction from crude kerosene acids and 5.2 g/kg bw for naphthenic acid fraction from mixed crude oils (Rockhold, 1955, as cited in Appendix C). An oral acute toxicity test in rats with a mixture of naphthenic acids isolated from Athabasca oil sands produced appetite suppression, hepatoxicity and cardiovascular effects with a single dose of 300 mg/kg. (Rogers, V.V., et al., Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355). An LD50 of 3.55 g/kg for mice was reported by Pennisi and Lynch, 1977 (as cited in Appendix C). |
| **Reliability** | : | (1) Reliable without restrictions, as assessed in Appendix C |
| **Reference** | : | Exxon, 1979. Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, as cited in Appendix C. |

### 5.1.2 ACUTE INHALATION TOXICITY

| | |
|---|---|
| **Type** | : |
| **Guideline/method** | : |
| **Species** | : |
| **Strain** | : |
| **Sex** | : |
| **Number of animals** | : |
| **Vehicle** | : |
| **Concentrations** | : |
| **Exposure time** | : |
| **LC50** | : |
| **Year** | : |
| **GLP** | : |
| **Test substance** | : |
| **Method** | : |
| **Method detail** | : |
| **Result** | : |
| **Remark** | : |
| **Reliability** | : |
| **Reference** | : |

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | Acute dermal LD50 with irritation |
| **Guideline/method** | : | |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand White |
| **Sex** | : | Male and female |
| **Number of animals** | : | 2 per sex |
| **Vehicle** | : | None – administered undiluted |
| **Doses** | : | 3.16 g/kg |
| **LD50** | : | > 3.16 g/kg |
| **Year** | : | 1979 |
| **GLP** | : | Unknown |
| **Test substance** | : | MRD-79-10 (raw naphthenic acid derived from kerosene) |

| | | |
|---|---|---|
| **Method** | : | |
| **Method detail** | : | The test substance was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil. Animals were then observed for mortality and toxic effects at 2 and 4 hours, and once daily thereafter. Body weight was recorded before and after the test. Dermal irritation was recorded at 1, 3, 7, 10 and 14 days. Mortality, toxicity and pharmacological effects were observed at 1, 2, 4, and 6 hours after dosing and once daily for 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology. |
| **Result** | : | No deaths occurred. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively. |
| **Remark** | : | |
| **Reliability** | : | (1) Reliable without restriction, as assessed in Appendix C |
| **Reference** | : | Exxon, 1979. Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, as cited in Appendix C |

### 5.2.1 SKIN IRRITATION

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Strain** | : | |
| **Sex** | : | |
| **Concentration** | : | |
| **Exposure** | : | |
| **Exposure time** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Classification** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | Moderately to severely irritating to rabbits. |
| **Remark** | : | See results of acute dermal LD50 study, described above. |
| **Reliability** | : | |
| **Reference** | : | |

### 5.2.2 EYE IRRITATION

| | | |
|---|---|---|
| **Type** | : | Eye irritation |
| **Guideline/method** | : | |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | Male and female |
| **Concentration** | : | |
| **Dose** | : | |
| **Exposure time** | : | |
| **Number of animals** | : | 3 per sex |

| | | |
|---|---|---|
| **Vehicle** | : | None – administered undiluted |
| **Classification** | : | |
| **Year** | : | 1979 |
| **GLP** | : | Unknown |
| **Test substance** | : | MRD-79-10 (raw naphthenic acid derived from kerosene) |
| **Method** | : | |
| **Method detail** | : | 0.1 mL of test substance was placed into the conjunctival sac of the eye of each of the six rabbits. The untreated eye served as a control. Animals were observed at 1 and 4 hours, and on days 1, 2, 3, 4 and 7. If a positive score was noted on day 7, ocular readings were scored on day 10. If an positive score was noted on day 10, observations were made on day 14. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system. |
| **Result** | : | One animal had a positive corneal score on days 1 and 2; one animal had a positive iris score at hours 1 and 4. All animals exhibited positive conjunctival scores at some point during the first three days of observation. By day 4, no animals showed positive scores. The test material was judged to be an irritant. In a later summary report, eye irritation was judged to be moderate. |
| **Remark** | : | |
| **Reliability** | : | (1) Reliable without restrictions, as assessed in Appendix C |
| **Reference** | : | Exxon, 1979. Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, as cited in Appendix C |

## 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | Oral 90-d subchronic toxicity test |
| **Guideline/method** | : | |
| **Species** | : | Rat |
| **Strain** | : | Wistar |
| **Sex** | : | Female |
| **Number of animals** | : | 12 per dose level |
| **Route of admin.** | : | Oral gavage |
| **Exposure period** | : | |
| **Frequency of treatment** | : | 1 dose/day, 5 days/week |
| **Post exposure period** | : | |
| **Dose** | : | 0.6, 6, or 60 mg/kg bw (aqueous solutions of naphthenic acids) |
| **Control group** | : | Yes (7 ml tap water) |
| **NOAEL** | : | 6 mg/kg/day |
| **LOAEL** | : | 60 mg/kg/day (5 doses per week) |
| **Other** | : | |
| **Year** | : | 2002 |
| **GLP** | : | Unknown |
| **Test substance** | : | Mixture of naphthenic acids (acyclic and 1-, 2-, 3-, and 4-ringed compounds, administered as sodium salt solutions) isolated from tailings pond water from Athabasca oil sands |
| **Method** | : | |
| **Method detail** | : | Animals were monitored daily. Changes in body weight, food consumption and behavioral or clinical signs recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Blood samples were similarly analyzed from cardiac punctures on day 91. Following euthanization, the liver, kidney, spleen, heart, lung and ovaries were examined. |
| **Result** | : | Significant physical, clinical, and pathological changes at a dose level of 60 mg/kg/day (5 doses per week). No significant adverse effects were seen at a dose level of 6 mg/kg/day. Several parameters suggested that the liver |

was the primary target organ in this study. Liver weight was increased 35% above control values in the high dose group. Body weight gain was also reduced 8-9% in this exposure group compared to controls. Plasma cholesterol was reduced and amaylase activity increased in the high dose group.

**Remark** :
**Reliability** : (2) Reliable with restriction. Only female rats were used and a limited number of organs examined.
**Reference** : Rogers et al. 2002. Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355.

## 5.5 GENETIC TOXICITY 'IN VITRO'

**Type** : Mutagenicity
**Guideline/method** : Ames assay
**System of testing** : Bacteria *in vitro*
**Species** : *Salmonella typhimurium*
**Strain** : TA100, TA1535, TA97, TA98
**Test concentrations** : 1 – 1000 ug/L depending upon strain
**Cytotoxic concentr.** :
**Metabolic activation** : With and without
**Year** : 1993
**GLP** : Yes
**Test substance** : Calcium naphthenate
**Method** :
**Method detail** : Activation was with induced male Sprague Dawley rat liver S9 and induced male Syrian hamster liver S9.
**Result** :
**Remark** : Sodium naphthenate was also negative in the *Salmonella* mutagenicity test, performed similarly.
**Reliability** : (1) Reliable without restriction
**Reference** : Study ID A21560 and Study ID 278018, National Toxicology Program (http://ntp-server.niehs.nih.gov)

**Type** : In vitro cytogenetics
**Guideline/method** :
**System of testing** :
**Species** :
**Strain** :
**Test concentrations** :
**Cytotoxic concentr.** :
**Metabolic activation** :
**Year** :
**GLP** : Yes
**Test substance** : Sodium naphthenate
**Method** :
**Method detail** :
**Result** : Negative results were obtained for chromosome aberrations, while positive results were obtained for Sister Chromatid Exchanges.
**Remark** :
**Reliability** : (1) Reliable without restriction
**Reference** : Study ID 058122, National Toxicology Program (http://ntp-server.niehs.nih.gov)

**Type** : Mutagenicity
**Guideline/method** :

| | | |
|---|---|---|
| **System of testing** | : | |
| **Species** | : | Mouse Lymphoma |
| **Strain** | : | L5178Y (TK+/TK-) |
| **Test concentrations** | : | 0.005 – 0.037 UL/ML |
| **Cytotoxic concentr.** | : | |
| **Metabolic activation** | : | none |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | Naphthenic acid, calcium salt (61789-36-4) |
| **Method** | : | Suspension Plate |
| **Method detail** | : | |
| **Result** | : | Positive |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | CCRIS (Record # 1169) () |

### 5.6 GENETIC TOXICITY 'IN VIVO'

| | |
|---|---|
| **Type** | : |
| **Guideline/method** | : |
| **Species** | : |
| **Strain** | : |
| **Sex** | : |
| **Route of admin.** | : |
| **Exposure period** | : |
| **Doses** | : |
| **Year** | : |
| **GLP** | : |
| **Test substance** | : |
| **Method** | : |
| **Method detail** | : |
| **Result** | : |
| **Remark** | : |
| **Reliability** | : |
| **Reference** | : |

### 5.8.2 DEVELOPMENTAL TOXICITY

| | |
|---|---|
| **Type** | : |
| **Guideline/method** | : |
| **Species** | : |
| **Strain** | : |
| **Sex** | : |
| **Route of admin.** | : |
| **Exposure period** | : |
| **Frequency of treatment** | : |
| **Duration of test** | : |
| **Doses** | : |
| **Control group** | : |
| **NOAEL maternal tox.** | : |
| **NOAEL teratogen.** | : |
| **Other** | : |
| **Other** | : |
| **Other** | : |
| **Year** | : |
| **GLP** | : |

| | | |
|---|---|---|
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

### 5.8.3 TOXICITY TO REPRODUCTION

| | | |
|---|---|---|
| **Type** | : | Dermal exposure |
| **Guideline/method** | : | |
| **In vitro/in vivo** | : | In vivo |
| **Species** | : | 12 male New Zealand White rabbits |
| **Strain** | : | |
| **Sex** | : | Male |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatment** | : | 5 days/week |
| **Duration of test** | : | |
| **Doses** | : | 2 ml undiluted material |
| **Control group** | : | 12 male |
| **Year** | : | 1984 |
| **GLP** | : | |
| **Test substance** | : | An over-based calcium naphthenate in mineral oil |
| **Method** | : | |
| **Method detail** | : | |
| **Results** | : | Results of the oral reproduction study are consistent with a one generation dermal reproduction study in male rabbits conducted on SAP 011, an overbased calcium naphthenate in mineral oil. A group of 12 male New Zealand White rabbits was dermally exposed to 2 ml of undiluted SAP 011 for 6 hours daily for 5 days each week over a 10-week period. Following the exposure period, each male rabbit was mated with two untreated female rabbits. Males were subsequently necropsied and their reproductive tracts examined macroscopically and microscopically. Female rabbits were necropsied on day 29 of gestation and examined for reproductive parameters. Study results showed no adverse effects on reproductive performance due to male exposure. There were no adverse signs of toxicity either systemically or at the site of application in treated males, as well as no pathological findings of the reproductive tract that could be related to SAP 011 exposure. |
| **Remark** | : | |
| **Reliability** | : | (2) Reliable with restrictions |
| **Reference** | : | Dix, K.M. and S.L. Cassidy. 1983. Toxicity studies on oil additives: one generation reproduction study in male rabbits repeatedly treated dermally with SAP 0111 for 10 weeks. External Report SBER.84.002. Shell Research Ltd. (NTIS No. OTS0507494) |

## 6.0 OTHER INFORMATION

### 6.1 Carcinogenicity

In a study in which calcium naphthenate was dermally administered to female mice (two times per day for two years), twelve epidermal and one dermal tumor at the treated sites were observed in eight of the exposed mice.

Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present. (Appendix C)

### 6.2 Skin sensitization

# 1. General Information

**ID** 7646-85-7

**Date** 2 Dec 2003

**201-16126B**

## 1.0 SUBSTANCE INFORMATION

| | | |
|---|---|---|
| **Generic Name** | : | Zinc chloride |
| **Chemical Name** | : | Zinc dichloride |
| **CAS Registry No.** | : | 7646-85-7 |
| **Component CAS Nos.** | : | |
| **EINECS No.** | : | 231-592-0 |
| **Structural Formula** | : | $ZnCl_2$ |
| **Additional description** | : | |
| **Molecular Weight** | : | 136.29 |
| **Synonyms and Tradenames** | : | Zinc (II) chloride; Butter of zinc; zinc butter; RTECS ZH1400000 |
| **References** | : | ATSDR, 2003 (Agency for Toxic Substances and Disease Registry, Draft Toxicological Profile for Zinc, September 2003) |

## 2.1 MELTING POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | 290 °C |
| **Decomposition** | : | |
| **Sublimation** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | 2 (reliable with restrictions): Source is well established data compendium. |
| **Reference** | : | O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ. |

## 2.2 BOILING POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | 732 °C |
| **Decomposition** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | 2 (reliable with restrictions): Source is well established data compendium. |
| **Reference** | : | O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ. |

## 2.3 DENSITY

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | 2.907 at 25°C |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | 2 (reliable with restrictions): Source is well established data compendium. |
| **Reference** | : | O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ. |

## 2.4 VAPOR PRESSURE

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | |
| **Decomposition** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Expected to be very low based on melting point and boiling point data. |
| **Reliability** | : | |
| **Reference** | : | |

## 2.5 PARTITION COEFFICIENT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Partition coefficient** | : | |
| **Log Pow** | : | |
| **pH value** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Not applicable – compound dissociates and ionizes in water |
| **Reliability** | : | |
| **Reference** | : | |

## 2.6.1 SOLUBILITY IN WATER

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | 4.32 X $10^6$ mg/L at 25 °C |
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |
| **PKa** | : | at °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Deg. products CAS#** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | 2 (reliable with restrictions): Source is well established data compendium. |
| **Reference** | : | O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ. |

## 2.7 FLASH POINT

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Value** | : | Not flammable |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

### 3.1.1 PHOTODEGRADATION

| | | | | |
|---|---|---|---|---|
| **Type** | | | | |
| **Guideline/method** | : | | | |
| **Light source** | : | | | |
| **Light spectrum** | : | | | |
| **Relative intensity** | : | based on | | |
| **Spectrum of substance** | : | lambda (max, >295nm) | : | |
| | | epsilon (max) | : | |
| | | epsilon (295) | : | |
| **Conc. of substance** | : | | at | °C |
| **DIRECT PHOTOLYSIS** | | | | |
| **Halflife (t1/2)** | : | | | |
| **Degradation** | : | % after | | |
| **Quantum yield** | : | | | |
| **INDIRECT PHOTOLYSIS** | | | | |
| **Sensitizer** | : | | | |
| **Conc. of sensitizer** | : | | | |
| **Rate constant** | : | | | |
| **Degradation** | : | | | |
| **Deg. product** | : | | | |
| **Year** | : | | | |
| **GLP** | : | | | |
| **Test substance** | : | | | |
| **Deg. products CAS#** | : | | | |
| **Method** | : | | | |
| **Method detail** | : | | | |
| **Result** | : | | | |
| **Remark** | : | Not applicable – the metal will not degrade | | |
| **Reliability** | : | | | |
| **Reference** | : | | | |

### 3.2.1 MONITORING DATA

| | | |
|---|---|---|
| **Type of measurement** | : | |
| **Media** | : | |
| **Concentration** | : | mg/l |
| **Substance measured** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

### 3.3.1 TRANSPORT (FUGACITY)

| | | |
|---|---|---|
| **Type** | : | |
| **Media** | : | |
| **Air** | : | % (Fugacity Model Level I) |
| **Water** | : | % (Fugacity Model Level I) |
| **Soil** | : | % (Fugacity Model Level I) |
| **Biota** | : | % (Fugacity Model Level II/III) |
| **Soil** | : | % (Fugacity Model Level II/III) |
| **Year** | : | |
| **Test substance** | : | |

| | | |
|---|---|---|
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

## 3.5 BIODEGRADATION

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Inoculum** | : | |
| **Concentration** | : | related to<br>related to |
| **Contact time** | : | |
| **Degradation** | : | (±) % after day(s) |
| **Result** | : | |
| **Kinetic of test subst.** | : | % (specify time and % degradation)<br>%<br>%<br>%<br>% |
| **Control substance** | : | |
| **Kinetic** | : | %<br>% |
| **Deg. product** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Deg. products CAS#** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Not applicable – the metal will not degrade |
| **Reliability** | : | |
| **Reference** | : | |

## 3.7 BIOCONCENTRATION

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Exposure period** | : | at °C |
| **Concentration** | : | |
| **BCF** | : | |
| **Elimination** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | |
| **Reliability** | : | |
| **Reference** | : | |

## 4.1 ACUTE TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | Acute |
| **Guideline/method** | : | Flow-through, freshwater |
| **Species** | : | Rainbow trout (*Onchorhynchus mykiss*) |
| **Exposure period** | : | 96 hr |
| **NOEC** | : | |
| **LC0** | : | |
| **LC50** | : | 93 – 0.815 μg Zn/L (depending on juvenile life-stage) |
| **LC100** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | No |
| **Year** | : | 1978 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | |
| **Method detail** | : | The toxicity of zinc chloride to four juvenile stages of rainbow trout (alvins, swim-up fry, parr, smolts) was determined in 96-h flow-through tests. |
| **Result** | : | LC50 values varied by life stage with the swim-up fry being the most sensitive. |
| **Remark** | : | The bioavailability and resultant aquatic toxicity of zinc chloride is affected by a variety of factors, including water hardness, pH, dissolved organic carbon and temperature. Reported 96-h LC50 values for zinc chloride (expressed as zinc) for various species of fish include 0.29 mg Zn/L and 0.42 mg Zn/L for bluegill (*Lepomis macrochirus*); 0.093 – 2.17 mg Zn/L for rainbow trout (*Onchorhynchus mykiss*), 0.45 - 2.25 mg Zn/L for common mirror-colored carp (*Cyprinus carpio*) and 1.70 mg Zn/L for sheepshead minnow (*Cyprinodon variegatus*) (U.S. EPA, ECOTOX database, 2003). |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Chapman, G.A. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of Chinook and steelheads. Trans. Am. Fish. Soc., 107(6):841-847. |

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Type** | : | Acute |
| **Guideline/method** | : | Flow-through, freshwater |
| **Species** | : | *Daphnia magna* |
| **Exposure period** | : | 48 hr |
| **NOEC** | : | |
| **EC0** | : | |
| **EC50** | : | 799 μg Zn/L |
| **EC100** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | |
| **Year** | : | 1982 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Flow-through |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | The bioavailability and resultant aquatic toxicity of zinc chloride is affected by a variety of factors, including water hardness, pH, dissolved organic carbon and temperature. Reported 48-h EC50 values for zinc chloride (expressed as zinc) for *Daphnia magna* include 0.33, 0.52, 0.66 and 0.80 |

| | | |
|---|---|---|
| | | mg Zn/L (U.S. EPA, ECOTOX database, 2003). For several crustaceans, including *Daphnia magna*, *Ceriodaphnia dubia*, and *Ceriodaphnia reticulata*, reported 48-h EC50 values ranged from 0.068 to 0.86 mg Zn/L, for zinc tested as zinc chloride or zinc sulfate. |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Attar, E.N. and E.J. Maly. 1982. Acute toxicity of cadmium, zinc, and cadmium-zinc mixtures to *Daphnia magna*. Arch. Environ. Contam. Toxicol., 11(3):291-296. |

## 4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

| | | |
|---|---|---|
| **Type** | : | Algal growth assay |
| **Guideline/method** | : | Static |
| **Species** | : | *Selenastrum capricornutum* |
| **Endpoint** | : | Growth |
| **Exposure period** | : | 96 hr |
| **NOEC** | : | |
| **LOEC** | : | |
| **EC0** | : | |
| **EC10** | : | |
| **EC50** | : | 44.7 µg Zn/L |
| **Limit test** | : | |
| **Analytical monitoring** | : | |
| **Year** | : | |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Microplate algal assay |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | The bioavailability and resultant aquatic toxicity of zinc is affected by a variety of factors, including water hardness, pH, dissolved organic carbon and temperature The reported 72-h EC50 for the marine diatom *Skeletonema costatum* was 0.142 mg Zn/L (U.S. EPA, ECOTOX database, 2003). |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Alaise, C., R. Legault, N. Bermingham, R. Van Coillie, and P. Vasseur. 1986. A simple microplate algal assay technique for aquatic toxicity assessment. Toxic. Assess., 1:261-281. |

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

| | | |
|---|---|---|
| **In vitro/in vivo** | : | |
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Number of animals** | : | |
| **Males** | : | |
| **Females** | : | |
| **Doses** | : | |
| **Males** | : | |
| **Females** | : | |
| **Vehicle** | : | |
| **Route of administration** | : | |
| **Exposure time** | : | |
| **Product type guidance** | : | |
| **Decision on results on acute tox. tests** | : | |
| **Adverse effects on prolonged exposure** | : | |
| **Half-lives** | : | 1st:<br>2nd:<br>3rd: |
| **Toxic behavior** | : | |
| **Deg. product** | : | |
| **Deg. products CAS#** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Zinc is an essential element in nutrition, and is important in membrane stability, in over 300 enzymes, and in the metabolism of proteins and acids. (WHO, 2001, Environmental Health Criteria 221, Zinc). Absorption of zinc in laboratory animals can vary from 10-40% depending upon nutritional status and other ligands in the diet. Absorbed zinc is mainly deposited in muscle, bone, liver, pancreas, kidney and other organs. The biological half-life of zinc ranges from 4 to 50 days in rats depending on the administered dose (WHO, 2001, Environmental Health Criteria 221, Zinc). Increases in zinc concentration in the bodies of experimental animals exposed to zinc are accompanied by reduced levels of copper, suggesting that some of the signs of toxicity ascribed to zinc may be caused by zinc-induced copper deficiency. Moreover, studies have shown that exposure to zinc alters the levels of other essential metals, including iron. Zinc deficiency in animals is characterized by a reduction in growth and cell replication, adverse reproductive and developmental effects, and reduced immunoresponsiveness. (WHO, 2001, Environmental Health Criteria 221, Zinc). |
| **Reliability** | : | |
| **Reference** | : | |

## 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | Oral |
| **Guideline** | : | Not specified |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male |
| **Number of animals** | : | 10 per dose group |
| **Vehicle** | : | Water |
| **Doses** | : | Not specified |
| **LD50** | : | 1,100 mg/kg b.w. as $ZnCl_2$ (95% C.I. = 661 – 1,830 mg/kg b.w.)<br>528 mg/kg b.w. as zinc (95% C.I. = 316 – 875 mg/kg b.w.) |
| **Year** | : | 1988 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Single doses administered intragastrically. |
| **Method detail** | : | Rats weighed 230 – 280 g. Solution concentrations were adjusted so that a 300–g rat received a 1 ml dose. Solutions were adjusted to a pH of between 6.0 and 7.0, using sodium biocarbonate when necessary. |
| **Result** | : | Acute LD50 values of zinc chloride were also determined using i.p administration in this study. The toxicity of zinc chloride to rats was much greater after i.p. administration with an LD50 of 58 mg/kg b.w. when expressed as $ZnCl_2$ (95% C.I. = 43 – 79) or 28 mg/kg b.w. when expressed as zinc (95% C.I. = 21 – 38). The much lower toxicity by the oral route of administration suggests a low rate of absorption of zinc chloride from the gastrointestinal tract. |
| **Remark** | : | Acute oral toxicity in rodents exposed to zinc compounds is low, and the level at which zinc produces no adverse effect in rats is approximately 160 mg/kg body weight (WHO, 2001, Environmental Health Criteria 221, Zinc). Of the compounds zinc nitrate, zinc sulfate, zinc chloride and zinc acetate, zinc acetate was the most toxic, with oral LD50 values of 237 mg Zn/kg bw (rat) and 86 mg Zn/kg bw (mouse). |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Domingo, J.L., J.M. Llobet, J.I. Paternain, and J. Corbella. 1988. Acute zinc intoxication: comparison of the antidotal efficacy of several chelating agents. Vet. Hum. Toxicol., 30(3): 224-228. |

| | | |
|---|---|---|
| **Type** | : | Oral |
| **Guideline/Method** | : | Not specified |
| **Species** | : | Mouse |
| **Strain** | : | Swiss |
| **Sex** | : | Male |
| **Number of animals** | : | 10 per dose group |
| **Vehicle** | : | Water |
| **Doses** | : | Not specified |
| **LD50** | : | 1,260 mg/kg b.w. as $ZnCl_2$ (95% C.I. = 775 – 2,300 mg/kg b.w.)<br>605 mg/kg b.w. as zinc (95% C.I. = 370 – 1,099 mg/kg b.w.) |
| **Year** | : | 1988 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Single doses administered intragastrically. |
| **Method detail** | : | Mice weighed 24 – 28 g. Solution concentrations were adjusted so that a 30–g mouse received a 0.21 ml dose. Solutions were adjusted to a pH of between 6.0 and 7.0, using sodium biocarbonate when necessary. |
| **Result** | : | Acute LD50 values of zinc chloride were also determined using i.p administration in this study. The toxicity of zinc chloride to mice was much |

| | | |
|---|---|---|
| | | greater after i.p. administration with an LD50 of 91 mg/kg b.w. when expressed as $ZnCl_2$ (95% C.I. = 57 – 146) or 44 mg/kg b.w. when expressed as zinc (95% C.I. = 27 – 69). The much lower toxicity by the oral route of administration suggests a low rate of absorption of zinc chloride from the gastrointestinal tract. |
| **Remark** | : | |
| **Reliability** | : | 2, reliable with restrictions: Comparable to guideline study with adequate documentation. |
| **Reference** | : | Domingo, J.L., J.M. Llobet, J.I. Paternain, and J. Corbella. 1988. Acute zinc intoxication: comparison of the antidotal efficacy of several chelating agents. Vet. Hum. Toxicol., 30(3): 224-228. |

### 5.1.2 ACUTE INHALATION TOXICITY

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Concentrations** | : | |
| **Exposure time** | : | |
| **LC50** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Zinc chloride is a primary ingredient in smoke bombs, resulting in respiratory injury. In a 10-minute inhalation study with rats, zinc chloride aerosol was lethal at concentrations as low as 940 mg $Zn/m^3$ (Risk Assessment for Zinc Metal, 2001, draft). |
| **Reliability** | : | |
| **Reference** | : | |

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | |
| **Guideline/method** | : | |
| **Species** | : | |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Doses** | : | |
| **LD50** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | |
| **Method detail** | : | |
| **Result** | : | |
| **Remark** | : | Zinc chloride is reported to cause moderate to severe skin irritation in the |

rabbit, guinea pig and mouse at 0.48 mg Zn/cm2 while zinc acetate at 7.2 mg $Zn/cm^2$ was reported to be irritating to the rabbit and mouse but caused no effects in the guinea pig (ATSDR, 1994, Toxicological Profile for Zinc).

**Reliability** :
**Reference** :

### 5.2.1 SKIN IRRITATION

**Type** :
**Guideline/method** :
**Species** :
**Strain** :
**Sex** :
**Concentration** :
**Exposure** :
**Exposure time** :
**Number of animals** :
**Vehicle** :
**Classification** :
**Year** :
**GLP** :
**Test substance** :
**Method** :
**Method detail** :
**Result** :
**Remark** : Zinc chloride, applied daily as a 1% aqueous solution in an open patch test for 5 days, was severely irritant in rabbits, guinea pigs and mice, inducing epidermal hyperplasia and ulceration. (Lansdown, 1991 as cited in WHO, 2001, Environmental Health Criteria 221, Zinc).

**Reliability** :
**Reference** :

### 5.2.2 EYE IRRITATION

**Type** :
**Guideline/method** :
**Species** :
**Strain** :
**Sex** :
**Concentration** :
**Dose** :
**Exposure time** :
**Number of animals** :
**Vehicle** :
**Classification** :
**Year** :
**GLP** :
**Test substance** :
**Method** :
**Method detail** :
**Result** :
**Remark** :
**Reliability** :
**Reference** :

## 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | 28-d Oral |
| **Guideline** | : | Not specified |
| **Species** | : | Rat |
| **Strain** | : | Wistar |
| **Sex** | : | Both male and female |
| **Number of animals** | : | 13 males; 17 females in treatment group |
| **Route of admin.** | : | Drinking water |
| **Exposure period** | : | 4 weeks |
| **Frequency of treatment** | : | Continuous |
| **Post exposure period** | : | None |
| **Doses** | : | 11.66 mg Zn/kg b.w./day in males and 12.75 mg Zn/kg b.w./day in females on average from 0.12 mg Zn/$cm^3$ in water |
| **Control group** | : | Yes |
| **NOAEL** | : | None |
| **LOAEL** | : | 12 mg Zn/kg b.w./day |
| **Other** | : | |
| **Year** | : | 1992 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | |
| **Method detail** | : | Two-month-old Wistar rats of both sexes received zinc chloride in their drinking water for a period of 4 weeks. Liquid consumption was monitored so that the average daily Zn exposure could be calculated. At study termination, rats were weighed, bled, and sacrificed. Hematological indices were determined on blood samples. |
| **Result** | : | Zinc treatment had no effect on the survival or body weight gain of exposed rats. Zinc treatment also had no appreciable affect on the composition of bone marrow cells. However, erythrocytes counts and hemoglobin levels in the peripheral blood were significantly decreased in Zn-exposed males and females compared to controls, while the numbers of leukocytes, neutrophils, and lymphocytes in male rats were increased compared to controls. |
| **Remark** | : | Long-term oral exposure to zinc compounds indicates the target organs of toxicity to be the hematopoeitic system in rats, ferrets and rabbits; the kidney in rats and ferrets; and the pancreas in mice and ferrets (WHO, 2001, Environmental Health Criteria 221, Zinc). Zinc acetate given to rats in water over three months yielded NOAEL values of 95 to 191 mg Zn/kg/d. During a 13-week exposure to zinc sulfate via the diet, NOAEL values for the rat ranged from 53 to 565 mg Zn/kg/day and for the mouse were 104 mg Zn/kg/d, based upon various parameters. (ATSDR, 2003, Draft Toxicological Profile for Zinc). |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Zaporowska, H. and W. Wasilewski. 1992. Combined effect of vanadium and zinc on certain selected haematological indices in rats. Comp. Biochem. Physiol., 103C: 143-147. |

| | | |
|---|---|---|
| **Type** | : | 13-week Oral |
| **Guideline/method** | : | Not specified |
| **Species** | : | Rat |
| **Strain** | : | Wistar |
| **Sex** | : | Male and female |
| **Number of animals** | : | 12 of each sex per treatment group |
| **Route of admin.** | : | Diet |
| **Exposure period** | : | 13 wk |

| | | |
|---|---|---|
| **Frequency of treatment** | : | Continuous |
| **Post exposure period** | : | None |
| **Doses** | : | 0, 300, 3,000, or 30,000 ppm in diet (equivalent to an average daily intake of 23.2, 234, or 2,514 mg $ZnSO_4$/kg/d in males and 24.5, 243, or 2,486 mg $ZnSO_4$/kg/d in females |
| **Control group** | : | Yes, for both males and females |
| **NOAEL** | : | 3,000 ppm in diet (equivalent to approximately 234 mg $ZnSO_4$/kg/d in males and 243 mg $ZnSO_4$/kg/d in females) |
| **LOAEL** | : | 30,000 ppm in diet (equivalent to approximately 2,514 mg $ZnSO_4$/kg/d in males and 2,486 mg $ZnSO_4$/kg/d in females) |
| **Other** | : | |
| **Year** | : | 1981 |
| **GLP** | : | No |
| **Test substance** | : | $ZnSO_4 \cdot 7H_2O$ |
| **Method** | : | |
| **Method detail** | : | Groups of male and female rats (12 each) were feed diets containing zinc sulfate for 13 weeks. Animals were observed daily for clinical signs of toxicity and weighed weekly. Feed and water intake was measured twice per week. Prior to study termination, blood samples were collected and analyzed for hematological and biochemical parameters. Following necropsy, gross pathological and histopathological examinations were conducted on selected target organs and tissues. Organs weights were also determined. |
| **Results** | : | No compound-related mortality was observed at any dose level. The only clinical signs of toxicity were behavioral (removal of chow from the feeding container) and confined to the highest feeding level (30,000 ppm). At the highest dose level, food consumption, water intake and growth were reduced, particularly in males. A moderate reduction in the total leukocyte count was observed in both sexes in the high dose groups, whereas males in this group also showed slightly decreased hematocrit and hemoglobin levels. GOT and GPT concentrations were decreased in all male groups but there was no dose-response trend. Total protein, cholesterol and calcium in the blood were decreased in high dose males, whereas only calcium was elevated in high dose females. Necropsy results indicated no remarkable gross lesions in rats at any dose level, although the weights (both absolute and relative) of the livers and kidneys of the males in the 30,00 ppm group showed a slight to moderate decrease. Histopathological examinations showed pancreatic lesions attributable to treatment in the high dose groups. Lesions consisted of degeneration and necrosis of the acinar cells, clarification of centroacinar cells, and interstitial fibrosis. |
| **Remark** | : | While not conducted on the zinc chloride salt, the results of this study on hydrated zinc sulfate are considered relevant for assessing the potential hazard of the chloride because both salts are soluble and expected to have a similar bioavailability and toxicity. In general, after oral or dermal exposure, the toxicities of all zinc compounds are comparable (ATSDR, 2003. Draft Toxicological Profile for Zinc). |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Maita, K., M. Hirano, K. Mitsumori, K. Takahashi, and Y. Shirasu. 1981. Subacute toxicity studies with zinc sulfate in mice and rats. J. Pesticide Sci., 6: 327-336. |
| **Type** | : | 13-week Oral |
| **Guideline/method** | : | Not specified |
| **Species** | : | Mouse |
| **Strain** | : | ICR (specific pathogen-free) |
| **Sex** | : | Male and female |

| | | |
|---|---|---|
| **Number of animals** | : | 12 of each sex per treatment group |
| **Route of admin.** | : | Diet |
| **Exposure period** | : | 13 wk |
| **Frequency of treatment** | : | Continuous |
| **Post exposure period** | : | None |
| **Doses** | : | 0, 300, 3,000, or 30,000 ppm in diet (equivalent to an average daily intake of 42.7, 458, or 4,927 mg $ZnSO_4$/kg/d in males and 46.4, 479, or 4,878 mg $ZnSO_4$/kg/d in females |
| **Control group** | : | Yes, for both males and females |
| **NOAEL** | : | 3,000 ppm in diet (equivalent to approximately 458 mg $ZnSO_4$/kg/d in males and 479 mg $ZnSO_4$/kg/d in females) |
| **LOAEL** | : | 30,000 ppm in diet (equivalent to approximately 4,927 mg $ZnSO_4$/kg/d in males and 4,878 mg $ZnSO_4$/kg/d in females) |
| **Other** | : | |
| **Year** | : | 1981 |
| **GLP** | : | No |
| **Test substance** | : | $ZnSO_4 \cdot 7H_2O$ |
| **Method** | : | |
| **Method detail** | : | Groups of male and female mice (12 each) were feed diets containing zinc sulfate for 13 weeks. Animals were observed daily for clinical signs of toxicity and weighed weekly. Feed and water intake was measured twice per week. Prior to study termination, blood samples were collected and analyzed for hematological and biochemical parameters. Following necropsy, gross pathological and histopathological examinations were conducted on selected target organs and tissues. Organs weights were also determined. |
| **Results** | : | Although there were no obvious clinical signs of toxicity, four of 12 males in the high dose (30,000 ppm) group died or were killed *in extremis*. One female fed at this level also died. Histological findings in these animals revealed impairment of the urinary tract and regressive changes in the exocrine gland of the pancreas. Food consumption, water intake, and growth were depressed in the high dose groups, with the greatest effects seen in males. Male and female mice in the 30,000 ppm group showed moderately reduced levels of hematocrit and hemoglobin compared to controls; the leukocyte counts in these males were also decreased moderately. Mice of both sexes in the high dose groups showed a slight to moderate decrease in total protein, glucose and cholesterol, and a moderate to marked increase in alkaline phosphatase and urea nitrogen. Additional findings included depressed GPT levels in females, increased blood calcium levels in females, and increased GOT levels in males. Gross pathological changes in the high-dose animals included marked emaciation, ischemic discoloration of the kidney and thyroid, atrophy of the pancreas, edematous thickening of the upper small intestine, slight splenomegaly, and ulcers of the fore-stomach. Histopathological lesions were observed in the pancreas (swollen nuclei, necrosis of acinar cells), upper intestine (proliferation of epithelial cells), fore-stomach (ulcerations), spleen (proliferation of erythropoietic immature cells), and kidney (regression of renal cortex in females). |
| **Remark** | : | Results were consistent with those in rats (see previous robust summary); however, the effects on mice were generally more severe at the same level (ppm) in the diet. Most likely this was due to the much higher dose levels of zinc sulfate in mice compared to rats (approximately double on a mg/kg/d basis) due to their smaller size and greater relative food intake. |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Maita, K., M. Hirano, K. Mitsumori, K. Takahashi, and Y. Shirasu. 1981. Subacute toxicity studies with zinc sulfate in mice and rats. . J. Pesticide |

Sci., 6: 327-336.

## 5.5 GENETIC TOXICITY - MUTAGENICITY

| | | |
|---|---|---|
| **Type** | : | Mutagenicity |
| **Guideline/method** | : | Rec-assay |
| **System of testing** | : | Bacteria *in vitro* |
| **Species** | : | *Bacillus subtilis* |
| **Strain** | : | H17 (rec+) and M45 (rec-) |
| **Test concentrations** | : | 0.05 M |
| **Cytotoxic concentr.** | : | Not determined |
| **Metabolic activation** | : | No |
| **Year** | : | 1975 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Kada et al., 1972. Mutation Res., 16:165-174. |
| **Method detail** | : | An 0.05 ml aliquot of a 0.05 M zinc chloride solution was tested. |
| **Result** | : | At the concentration tested, there was no inhibition of either the rec+ or rec- strain of *Bacillus subtilis*, suggesting that zinc chloride did not cause DNA damage. |
| **Remark** | : | In 11 separate in vitro studies with zinc chloride or zinc sulfate, negative results were reported with the exception of two ambiguous results and one weakly positive result. (Risk Assessment for Zinc Metal, 2001, draft). Genotoxicity studies in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenicity following zinc exposure (ATSDR, 2003 Draft Toxicological Profile for Zinc). The results of short-term genotoxicity assays for zinc are equivocal. Responses in mutagenicity assays are thought to depend on the form (e.g., inorganic or organic salt) of the zinc tested (U.S. EPA, 2003, Integrated Risk Information System (IRIS) Summary for Zinc and Compounds). |
| **Reliability** | : | 2 (reliable with restrictions): Acceptable study with adequate documentation. |
| **Reference** | : | Nisioka, H. 1975. Mutagenic activities of metal compounds in bacteria. Mutation Res., 31: 185-189. |

| | | |
|---|---|---|
| **Type** | : | Mutagenicity |
| **Guideline/method** | : | Microscreen assay |
| **System of testing** | : | Bacteria *in vitro* |
| **Species** | : | *Escherichia coli* |
| **Strain** | : | $WP_s(\lambda)$ |
| **Test concentrations** | : | Not specified |
| **Cytotoxic concentr.** | : | >1 mM |
| **Metabolic activation** | : | No |
| **Year** | : | 1987 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Rossman et al., 1984. Environ. Mut., 6:59. |
| **Method detail** | : | |
| **Result** | : | Negative for Trp+ reversion, λ Prophage induction and WP2 comutagenenesis |
| **Remark** | : | |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Rossman, T.G., J.T. Zelikoff, S. Agarwal, and T.J. Kneip. 1987. Genetic |

toxicology of metal compounds: an examination of appropriate cellular models. Toxicol. Environ. Chem., 14:251-262.

| | | |
|---|---|---|
| **Type** | : | Mutagenicity |
| **Guideline/method** | : | L5178Y/TK somatic cell point mutation assay |
| **System of testing** | : | Cultured mouse lymphoma cells – *in vitro* |
| **Species** | : | Mouse |
| **Strain** | : | L5178/TK$^{+/-}$ |
| **Test concentrations** | : | 1.21 – 12.13 µg/ml |
| **Cytotoxic concentr.** | : | Not determined |
| **Metabolic activation** | : | No |
| **Year** | : | 1980 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Clive et al., 1972. Mutation Res., 16:77-87. |
| **Method detail** | : | |
| **Result** | : | Zinc chloride was not mutagenic under the test conditions. |
| **Remark** | : | |
| **Reliability** | : | 2 (reliable with restrictions): Acceptable study with adequate documentation. |
| **Reference** | : | Amacher, D.E. and S.C. Paillet. 1980. Induction of trifluorothymidine-resistant mutants by metal ions in L5178Y/TK+/- cells. Mutation Res., 78: 279-288. |

## 5.6 GENETIC TOXICITY - CLASTOGENICITY

| | | |
|---|---|---|
| **Type** | : | Chromosomal aberrations in bone marrow cells |
| **Guideline/method** | : | *In vivo* |
| **Species** | : | Mouse |
| **Strain** | : | C57B1 |
| **Sex** | : | Male |
| **Route of admin.** | : | Diet |
| **Exposure period** | : | One month |
| **Doses** | : | 0.5% Zn in feed |
| **Year** | : | 1979 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | |
| **Method detail** | : | 8-week-old mice kept on a normal (1.1% calcium) or low-calcium (0.03%) diet were exposed for one month to zinc chloride (0.5% Zn). After test termination, the bone marrow cells (50 metaphases/animal) from 10 animals were assayed for chromosomal aberrations. |
| **Result** | : | The body weights of mice fed zinc in the diet, either with normal or low calcium, were significantly reduced compared to their respective controls. Zinc treatment caused a significant increase in cells with structural aberrations (primarily dicentric chromosomes) for mice on low calcium diets. Aberrations were also increased in Zn-treated mice with normal calcium diets, but the increase was not statistically significant. |
| **Remark** | : | Studies on the induction of chromosome aberrations in bone marrow cells harvested from animals exposed to zinc compounds have yielded equivocal results. Increased aberrations have been seen in rats after oral exposure to zinc chloride in water (249 mg/L for 14 days) and in mice given intraperitoneal injections of zinc chloride (2-5 mg/kg as zinc chloride). In contrast, other studies have produced negative findings or have suggested that the induction of aberrations is contingent upon concomitant calcium deficiency. Negative results have been reported in the mouse micronucleus |

test (i.p. injection of zinc sulfate) and in the dominant lethal mutation assay with mice (i.p. injection of zinc chloride at 15 mg/kg). (WHO, 2001, Environmental Health Criteria 221, Zinc).

**Reliability** : 2 (reliable with restrictions): Acceptable study with adequate documentation.

**Reference** : G. Deknudt and G.B. Gerber. 1979. chromosomal aberrations in bone-marrow cells of mice given a normal or a calcium-deficient diet supplemented with various heavy metals. Mutation Res., 68:163-168.

### 5.8.2 DEVELOPMENTAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | Teratogenicity |
| **Guideline** | : | Not specified |
| **Species** | : | Mouse |
| **Strain** | : | CF-1 albino |
| **Sex** | : | Female |
| **Route of admin.** | : | Intraperitoneal |
| **Exposure period** | : | Day 8, 9, 10, or 11 of gestation |
| **Frequency of treatment** | : | Single dose |
| **Duration of test** | : | To gestation Day 18 |
| **Doses** | : | 12.5, 20.5, or 25 mg $ZnCl_2$/kg |
| **Control group** | : | Yes (distilled water only) |
| **NOAEL maternal tox.** | : | 12.5 mg $ZnCl_2$/kg |
| **NOAEL teratogen.** | : | 12.5 mg $ZnCl_2$/kg |
| **Other** | : | |
| **Other** | : | |
| **Other** | : | |
| **Year** | : | 1977 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | |
| **Method detail** | : | Gravid female mice were given an i.p. injection of either 12.5, 20.5 or 25 mg $ZnCl_2$/kg on Day 8, 9, 10, or 11 of gestation. Following the respective treatments, the mice were allowed to continue their gestation uninterrupted until Day 18 (one day prior to expected delivery), when each pregnant mouse was sacrificed. The number of fetuses and resorption sites (metrial glands) was determined and recorded. Each fetus was then weighed, sexed, and examined for external defects. Every other fetus was processed for skeletal examination by the method of Staples and Schnell (1964). |
| **Result** | : | Zinc chloride, when administered in doses of 20.5 and 25 mg/kg, produced significant incidences of skeletal defects in fetuses as compared to those observed in the water-treated group on Day 11. Both doses also resulted in mortality of gravid females. The majority of defects involved the rib cage and included a ripple rib anomaly; however, the zinc salt failed to produce a significant incidence of soft tissue anomalies with either treatment regimen. As the dosage of $ZnCl_2$ was reduced, maternal and fetal toxicity, relative fetal weights, and the incidences of skeletal anomalies were correspondingly decreased. Maternal toxicity and incidences of skeletal anomalies were greatest when doses were administered on Day 11 of gestation. Zinc chloride, given at 12.5 mg/kg on day 11 of gestation, induced nonsignificant incidences of both skeletal and soft tissue defects compared to controls. No deaths were observed in the gravid females and no ripple ribs were observed in their fetuses. |
| **Remark** | : | Developmental toxicity data for several zinc compounds are available. Second-generation mice (from mothers fed zinc carbonate) exposed to high doses of zinc throughout the gestation, lactation, and postweaning periods had elevated levels of zinc in their bones, decreased blood copper levels, |

lowered hematocrit values and reduced body weights. The offspring of pregnant rats fed zinc carbonate (500 mg Zn/kg) did not demonstrate any increase in the incidence of malformations. (WHO, 2001, Environmental Health Criteria 221, Zinc). Several developmental toxicity studies have been conducted with zinc sulfate on mice, rats, hamsters and rabbits, in general accordance with OECD Guideline 414; however, the form of the zinc sulfate was not specified. Depending upon the form that was used, the calculated NOAEL values ranged from 6.8 mg Zn/kg bw for the mouse to 35.2 mg Zn/kg bw for the hamster. (Risk Assessment for Zinc Metal, 2001, draft).

**Reliability** : 2 (reliable with restrictions): Comparable to guideline study with adequate documentation.

**Reference** : Chang, C-H., D.E. Mann, and R.F. Gautieri. 1977. Teratogenicity of zinc chloride, 1,10-phenanthroline, and a zinc-1,10-phenanthroline complex in mice. J. Pharm. Sci., 66:1755-1758.

### 5.8.3 TOXICITY TO REPRODUCTION

| | | |
|---|---|---|
| **Type** | : | Single-generation pilot breeding study |
| **Guideline** | : | Not specified |
| **In vitro/in vivo** | : | In vivo |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley SDTM |
| **Sex** | : | Both male and female |
| **Route of admin.** | : | Oral gavage |
| **Exposure period** | : | Males: Prior to cohabitation (77 d) and during cohabitation (21 d)<br>Females: Prior to cohabitation (77 d), during cohabitation (21 d), and throughout gestation (21 d) and lactation (21 d). |
| **Frequency of treatment** | : | 7 days/week |
| **Duration of test** | : | 140 days (20 wk) |
| **Doses** | : | 0, 7.5, 15, and 30 mg $ZnCl_2$/kg/d |
| **Control group** | : | Yes |
| **Year** | : | 2001 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | Single generation breeding study |
| **Method detail** | : | Male and female rats (10 each per treatment) were administered 0.0, 7.5, 15.0, or 30.0 $ZnCl_2$ for 77 days prior to mating. At the end of the pre-mating period, males and females were paired within the same dose groups. Dosing was continued for both sexes throughout mating. All males were euthanized at the conclusion of mating, weighed, necropsied, and examined for morphological changes. Dosing was continued for females throughout gestation and lactation. Pregnant females were allowed to deliver their offspring naturally. Litter sizes were standardized on day 4 after birth to 4 of each sex. At day 21 of lactation, all $F_0$ females were sacrificed, necropsied, and examined for morphological changes. The evaluation of reproductive performance included fertility, viability index, weaning index, litter size, and the body weight of pups on days 0, 4, 7, 14, and 21 of lactation. |
| **Results** | : | The fertility indices in all dose groups were significantly lower than in the control group, but did not show a dose-response relationship. Pup viability indices on days 0 and 4 for the high-dose group were significantly lower than those of the control group. The body weights of pups in the highest dose group on days 14 and 21 were significantly lower than those in the control group. There were no effects on weaning indices or sex ratios. Overall, the results suggested that $ZnCl_2$ has only mild effects on rat reproductive performance up to 30 mg/kg/d. In addition, there were no |

| | | |
|---|---|---|
| | | significant treatment-related changes observed in any of the clinical pathology parameters that were evaluated. All histopathologic effects related to treatment were mild. Those in the reproductive organs were confined to males only and according to the authors probably precluded any adverse effects upon reproduction. |
| **Remark** | : | The effects on reproduction of other zinc compounds have also been studied. The LOAEL for serious reproductive effects in female rats was 200 and 250 mg Zn/kg/d from exposure to zinc sulfate and zinc carbonate, respectively, in the diet. (ATSDR, 2003, Draft Toxicological Profile for Zinc). |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Khan, A.T., A. Atkinson, T.C. Graham, M. Green, S. Ali, S.J. Thompson, and K.F. Shireen. 2001. Effects of low levels of zinc on reproductive performance of rats. Environ. Sci. (Tokyo), 8(4): 367-381. |
| **Type** | : | Sperm chromatin structure |
| **Guideline** | : | None |
| **In vitro/in vivo** | : | In vivo |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male |
| **Route of admin.** | : | Diet |
| **Exposure period** | : | 8 weeks |
| **Frequency of treatment** | : | Continuous |
| **Duration of test** | : | 8 weeks |
| **Doses** | : | 4, 12, or 500 mg Zn/kg of diet (ppm) |
| **Control group** | : | No |
| **Year** | : | 1993 |
| **GLP** | : | No |
| **Test substance** | : | Zinc chloride |
| **Method** | : | |
| **Method detail** | : | Three-week old male rats (10 per group) were fed experimental diets with concentrations of zinc considered to be deficient (4 mg/kg), adequate (12 mg/kg) or excessive (500 mg/kg). After 8 weeks of feeding, animals were sacrificed to obtain testicular germ cells and epididymal sperm. Flow-cytometric procedures were used to determine effects on rat testicular development, including integrity of caudal epididymal sperm chromatin structure defined as the susceptibility of DNA to denaturation *in situ*. |
| **Results** | : | Rats fed the zinc deficient (4 ppm) diet demonstrated significant deviations in the ratio of testicular cell types present, including a reduction of S phase and total haploid cells. In addition, approximately 50% of epididymal sperm has a significant decrease in resistance to DNA denaturation *in situ*. Rats fed either a Zn-adequate or Zn-excess diet did not demonstrate an abnormal testicular cell type ratio. Excess Zn had a negative effect on chromatin structure, but much less than that of Zn deficiency. |
| **Remark** | : | Rats fed zinc chloride daily over an 8 week period demonstrated altered sperm chromatin structure with a LOAEL of 25 mg Zn/kg/d. |
| **Reliability** | : | 2 (reliable with restrictions): Comparable to guideline study with adequate documentation. |
| **Reference** | : | Evenson, D.P., R.J. Emerick, L.K. Jost, H. Kayongo-Male, and S.R. Stewart. 1993. Zinc-silicon interactions influencing sperm chromatin integrity and testicular cell development in the rat as measured by flow cytometry. J. Anim. Sci., 71:955-962. |

## 6.0 OTHER INFORMATION

### 6.1 Carcinogenicity

No adequate experimental evidence has been found to indicate that zinc salts administered orally or parenterally are tumorigenic. (WHO, 2001, Environmental Health Criteria 221, Zinc).